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Studies on the Mechanism of the Autoxidation

of

Unsaturated Fatty Acid Esters

Final Report on Contract No. N8ONR-66209(NR 055-251)

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This report summarizes research of the past two years on the basic reactions the autoxidation of unsaturated fatty acid esters carried out under contract with the Office of Naval Research. For a comprehensive review of previous work autoxidation, the reader is referred to papers by workers at the research laboratories of the British Rubber Producers Association (5,6,7,8,9,10), by Lundberg et al. (18), Swern et al. (23), Hilditch (13), and the Symposium on Oxidation published in the Transactions of the Faraday Society (24).

In general, the elucidation of the mechanism of autoxidation has stemmed from kinetic data, and from such information as has been available on the products of the reaction. However, it is evident from an examination of the literature that while considerable information about fat oxidation has been obtained along certain lines in the past decade, many important areas of the problem have yet to be studied. In particular, further information is needed about the structure of the peroxides, the decomposition of the peroxides, and the products of such decomposition.

Most studies of the products of the autoxidation of fatty acid esters have been handicapped by the lack of a suitable method for the quantitative isolation of the reaction products. Further, until recently it was not generally realized that peroxides interfere in many of the standard methods of chemical analyses of fats.

Thus, the first objective of this work consisted in the development of a countercurrent solvent method for the quantitative separation of the products of autoxidation in a high degree of purity. Then, information on the mechanism of the reaction was derived from physical and chemical studies of the structures of the products formed in the oxidation of highly purified methyl oleate, linoleate, and linolenate under various experimental conditions.

Two papers have been published on the results of the studies undertaken in this project. These are entitled: "Preparation of Peroxide Concentrates from Autoxidized Fatty Acid Esters" (20), and "Structure of Hydroperoxides Obtained from Autoxidized Methyl Linoleate" (21). In addition, five other manuscripts covering the remainder of the work are now being prepared for publication, the results of which are summarized in this report. These are entitled:
I. Evidence for Hydroperoxide Formation in the Autoxidation of Methyl Linolenate,
II. Studies on the Products of the Lipoxidase Catalyzed Oxidation of Sodium Linoleate and Sodium Linolenate, III. Kinetics of the Decomposition of the Peroxides of Autoxidized Methyl Linoleate and Methyl Oleate, IV. Photochemical Oxidation of Methyl Oleate and Methyl Linoleate with and without Chlorophyll,
V. Kinetics of the Photochemical Oxidation of Fatty Acid Esters with Chlorophyll. Partially completed work consists of studies on the isolation of the products of the autoxidation of methyl oleate, methyl linoelaidate, and methyl cis,trans linoleate. Also, a new colorimetric method for the estimation of organic peroxides is being developed.

Materials and Methods

Highly purified fatty acid esters were employed in all of the studies unless otherwise specified. Methyl oleate was isolated from olive oil by low temperature crystallization followed by fractional distillation, and had an iodine value (Wijs) of 85.2 (theoretical 85.6). The methyl linoleate and linolenate were prepared from their corresponding bromostearic acids (purified by repeated recrystallizations), and had iodine values of 173 (theoretical 172.4) and 260 (theoretical 260.4) respectively. The ultraviolet absorption spectra showed that all of these esters were essentially free of conjugated materials.

Autoxidation: In order to minimize secondary reactions, the autoxidations were generally conducted at relatively low temperatures. However, since methyl oleate autoxidizes very slowly at low temperatures, the autoxidation of this ester in the experiments reported herein was generally carried out by bubbling oxygen through 100 gram samples in a bath at 50° C. The autoxidation of methyl linoleate and linolenate was accomplished by keeping approximately 100 gram samples in the dark at $0-2^\circ$ C. in loosely stoppered Erlenmyer flasks. The flasks were uncovered and shaken at frequent intervals to replenish the supply of dissolved oxygen. The progress of the oxidation in all cases was followed by periodic determination of the peroxide values.

Lipoxidase catalyzed oxidation of fatty acids and fatty acid esters: Crude lipoxidase preparations were used in these studies, and were obtained by aqueous extraction of defatted soybean flour. Fifteen parts by weight of defatted soybean flour was stirred with 100 parts of a solution consisting of 13 g. of sodium acetate and 0.76 ml. of glacial acetic acid per 100 ml. of distilled water (pH 5-6). After stirring for at least one hour the suspension was filtered. The filtrate containing the enzyme was adjusted to pH 7 with aqueous potassium hydroxide and stored in the cold until used.

Batch oxidations at 0° C. were conducted in the following manner: The substrate, consisting of 25 g. of sodium soap dissolved in a sufficient amount of 0.1 N ammonium hydroxide-ammonium chloride buffer (pH 9) to give a volume of 500 ml., was cooled to 0° C. and saturated with oxygen. 170 ml. of the lipoxidase extract, which also was cooled to 0° C. and saturated with oxygen, was added to the substrate and the mixture stirred vigorously under a blanket of oxygen. At 7 and 14 minute intervals an additional 120 ml. of cooled lipoxidase extract saturated with oxygen, and 240 ml. of buffer were added to the reaction. The concentration of the soap at this point was approximately 20 mg. per ml., and the concentration of the lipoxidase was approximately 15 units per ml. Since it was not desirable to carry the oxidation beyond about 0.1 mole of oxygen per mole of fatty acid, the reaction was stopped at the end of 20 minutes by adding 95% ethanol. The soap-lipoxidase solution was acidified with 50% HCl and the fatty acids recovered by repeated extraction with Skellysolve F.

Oxygen absorption and enzyme and peroxide relationships were obtained at room temperature in a specially designed apparatus in which 15 grams of soaps could be oxidized to about 10% levels.

Photo-oxidation of chlorophyll: Crude chlorophyll was obtained from fresh spinach leaves and freed from other plant pigments in order to retain only chlorophylls A and B. The methods are described in detail elsewhere (14).

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30% solutions of methyl oleate or linoleate in heptane with added chlorophyll were oxidized at -1 to -2° C. under irradiation with visible light. In cases where products for structure analysis were prepared, the oxidation was conducted in open vessels. In kinetic studies, where the rate of oxygen uptake was measured, closed systems were used.

In order to compare the effect of intermittent radiation and continuous radiation on the photo-chlorophyll oxidation, a rotating sector device was used which is also described in detail elsewhere (14).

Decomposition of peroxides: Small samples (1 ml.) of oxidized ester were sealed in 25 ml. round bottom flasks after a degassing period of about 36 hours at less than 10 microns. By means of a specially constructed manifold, 30 samples could be degassed at the same time. The samples were decomposed by suspending the flasks in an oven maintained at constant temperature. Generally 5 samples were used for each experiment.

Reduction of peroxides: The reduction was carried out in a 0.5% stannous chloride solution of diethyl ether or ethyl alcohol. The samples were dissolved in a sufficient amount of the solution to give 3 to 5 times the amount of stannous chloride necessary for the quantitative reduction of the peroxides. The reduction was essentially complete after a period of $2\frac{1}{2}$ hours, during which time nitrogen was continuously bubbled through the solution to provide an inert atmosphere. The unreacted stannous chloride and the metastannic acid produced was removed by extraction from a petroleum ether-ethyl ether mixture with 5% HCl or by precipitation with ammonium hydroxide followed by filtration.

Analytical micro molecular distillation: The micromolecular still of Paschke and Wheeler (22) was used to measure and separate monomer, dimer, and higher polymer fractions of a number of reduced peroxide concentrates. The monomer was collected at 150° C., the dimer at 250° C., and the higher polymer fraction remained in the residue and was calculated by difference.

Countercurrent fractionation: The oxidized fraction of autoxidized esters was fractionated in some experiments by the countercurrent method of extraction previously described (20). Weight distribution, and chemical and spectral analyses were made on the individual fractions in these experiments.

Hydrogenation: Quantitative hydrogenations were conducted on a semimicro scale. In the first analyses a platinum catalyst was used, and the reaction was carried out in dioxane. Later determinations were made using a modified apparatus immersed in a water bath to avoid temperature fluctuation. Silicone manometer fluid was used in place of mercury to indicate pressure changes, and the reaction was carried out in 95% alcohol using a palladium catalyst. By this procedure the error in duplicate determinations was reduced to +0.5%.

Molecular weight: The cryoscopic method of Wilson and Heron (28) was used to determine molecular weights.

Infrared absorption spectra were obtained using a Beckman I.R. 2 model spectrophotometer equipped with special slit drives for rock salt and lithium fluoride prisms (26). The spectra was determined on solutions of samples in either carbon disulphide or tetrachloroethylene, depending on the region of the spectra under examination.

Ultraviolet absorption spectra were obtained using a Beckman D.U. model spectrophotometer on solutions of the samples in alcohol.

Peroxide value. Peroxide content was determined according to a modified iodometric procedure, previously described, (20) in which air was excluded during all critical stages of the procedure.

Esterification: Esterification of reduced peroxide concentrates were carried out by means of diazomethane in anhydrous ethyl ether at 0° C.

Hydroxyl number: The acetic anhydride-pyridine macro-method of Ogg, Porter, and Willits (19) was modified for use on a semi-micro scale. Thymolthalein was substituted for their mixed indicator to give a sharper end-point with the more dilute reagents.

Epoxy (15) and carbonyl oxygen (11) were determined by standard procedures.

Autoxidation of Methyl Oleate

The oxidized fractions of two samples of methyl oleate autoxidized to peroxide values of 800 and 1695 me./kg. respectively have been analyzed. The chemical analyses of the reduced and unreduced concentrate are shown in Table I. The ultraviolet and infrared absorption spectra are shown in Figures 1 and 2 respectively.

It is evident from studies conducted on the decomposition of oleate peroxide, reported further on in this report, that some decomposition undoubtedly occurred under the conditions of autoxidation employed in these studies. There is some evidence for this in the analysis by the low peroxide values (88% of theory), and the ultraviolet absorption spectra of the reduced concentrates. Furthermore, there is some evidence to indicate that the peroxide fraction doesn't consist entirely of hydroperoxides. The distillation analyses on concentrate II, Table I, rules out the formation of polymeric peroxides, and thus the presence of cyclic monomeric peroxides, especially in concentrate II, must be considered highly plausible. At present, it is not known whether these cyclic peroxides form concurrently with the hydroperoxides or result from their further reaction. Experiments are in progress which, it is hoped, will throw light on this important question. The structures existing in the hydroperoxide fraction of autoxidized methyl oleate have never been fully determined. Information on these is important to a complete understanding of the mechanism of autoxidation of methyl oleate.

Autoxidation of Methyl Linoleate

Some work on this project on the autoxidation of methyl linoleate has already been published, but another sample of methyl linoleate autoxidized at 0° C. to a peroxide value of 1020 me./kg. of ester has also been studied. Chemical and ultraviolet spectral analyses on the stannous chloride reduced concentrate and distilled monomer thereof are shown in Table 2.

It was shown in initial studies (21) that at least 90% of the initially formed peroxides in the autoxidation of methyl linoleate at 0° C. were of the conjugated cis,trans type. Results of the analyses on the concentrate reported here showed that even when the autoxidation was carried to peroxide values as high as 1000 me./kg. at 0° C., the major portion of the oxidized fraction consisted of cis,trans conjugated hydroperoxides (Figure 3). Since some secondary reactions occurred at this level of autoxidation, as indicated by the presence

of polymers in the oxidized fraction, it appeared that the formation of trans, trans conjugated hydroperoxides was related to the temperature of the reaction. Other studies showed that trans-trans peroxides are formed predominantly in photo-oxidations, with or without photochemical pigments. These observations indicate that the inversion of cis double bonds to trans configuration is independent of the primary oxidation reaction and may, indeed, occur after the peroxides are formed.

Autoxidation of Methyl Linolenate

Except for the recent work of Pugger et al., (12) most workers in the field of autoxidation assumed that the basic reactions of the autoxidation of methyl linolenate were similar to those for methyl oleate and methyl linoleate. However, there is no report of the isolation of hydroperoxides (considered to be the initial reaction products in the autoxidation of oleate and linoleate) from autoxidized methyl linolenate. Pugger et al., (12) concluded that little, if any, hydroperoxides accumulated in autoxidized methyl linolenate, and that the course of autoxidation differed significantly from that of oleate and linoleate. In this study convincing evidence (Tables 3, 4 and 5 and Figures 4, 5, 6, 7, 8, and 9) was obtained for the presence of appreciable amounts (50-60%) of hydroperoxides in methyl linolenate autoxidized at 0° C. to peroxide values of 600-760 me./kg. of ester. Furthermore, hydroperoxide concentrates with an estimated purity of 80-85%, and consisting of 40-44% of the oxidized fraction were isolated. There was no evidence in these concentrates of double bond configurations other than those of the cis,trans conjugated type. This parallels the situation in the case of methyl linoleate and is in accord with the deduction of Riemenschneider that when a double bond shifts it invariably assumes a trans configuration. Further experiments are planned to determine the effect of temperature on the structure of the hydroperoxides formed.

Lipoxidase Catalyzed Oxidation of Sodium Linoleate

Progress in the elucidation of the mechanism of the lipoxidase catalyzed oxidation of linoleate has been slower than in the case of ordinary autoxidation. One reason for this perhaps is that the reaction is complicated by several additional factors.

Chiefly from a consideration of the kinetics of the reaction, and apparent differences in the products formed, Tappel et al., (25) proposed a simple enzymatic mechanism in accord with generally accepted concepts of enzyme action.

Except for Bergstrom (3) who compared the products of lipoxidase catalyzed oxidation with those catalyzed by copper, it has generally been considered (4, 17) that the structures of the peroxides formed in the presence of lipoxidase differed from those formed in autoxidation. This conclusion was based almost entirely on the apparent relatively high molecular extinction coefficient of the peroxides. However, the analyses were made on the basis of oxygen absorption or on the recovered free acids; the products of oxidation were not isolated.

Our studies on the kinetics of the reaction gave results conforming to the characteristic of enzymatic reactions in agreement with the findings of Tappel et al., (25) and provided new information concerning the structures of the initial products.

The oxidized fractions of various samples oxidized at both 0° and $24-26^\circ$ C. were isolated by countercurrent extraction and analyzed (Table 8). In Figure 19, the infrared spectra of a peroxide concentrate obtained from a lipoxidase catalyzed oxidation is compared to one isolated from a sample of autoxidized methyl linoleate. These spectral absorption curves showed that the geometric configuration of the diene conjugation was identical in both types of oxidation. In Figure 20, the infrared spectra of a number of combined fractions obtained from countercurrent distribution of the oxidized material of linoleic acid is shown. The nature of the material that has the strong absorption band at 9.56 microns in the hydroxy stretching region is not known, but since it does not appear in all the fractions, it apparently is not associated with conjugated diene structures. The possibility that it is due to a glycolic structure is being investigated.

The ultraviolet spectra of the original samples of lipoxidase catalyzed linoleate (measured on the free acids) and the oxidized fraction separated from the recovered acids by countercurrent extraction are shown in Figure 21. If all the diene conjugation in these concentrates (Table 8) were attributed to the stable hydroperoxides they would have specific extinction coefficients of about 88-89. This value is considerably higher than for hydroperoxides produced in 0° C. autoxidations of linoleate, and even higher than for a pure cis,trans conjugated methyl octadecadienoate hydroperoxide. Thus, since the infrared spectra (Figures 19 and 20) of peroxides produced in this type of oxidation showed that virtually all of the conjugation existed in the cis,trans configuration similar to that produced in 0° C. autoxidation of linoleate, it is evident that products other than hydroperoxides are present in the concentrate which also contain conjugated dienes.

At present no conclusions can be made concerning the conjugated double bonds of the peroxides produced in room temperature oxidations. However, it is suspected that the initially produced peroxides at this temperature also contain a cis,trans conjugated configuration of double bonds. The linoleate peroxide concentrate shown in Table 8 appeared to contain about 40% of trans,trans and 60% of the cis,trans conjugated isomers. On the other hand, another peroxide concentrate (97.5%) obtained in the course of developing a method for isolating essentially pure hydroperoxides from this type of oxidized material, contained mostly trans,trans conjugation. This concentrate had a specific extinction coefficient of 79.0 at 234 millimicrons. Thus, it appears that the conditions of oxidation and handling have an influence on the structure of the peroxides.

Most significant also was the fact that polymers were produced in the lipoxidase-catalyzed oxidation of corn oil soaps. At present, the reactions involving polymer formation in lipoxidase catalyzed oxidation are not known. Presumably, in both autoxidation and lipoxidase catalyzed oxidation, some polymers could form from the intact peroxides. More likely, however, the polymers are formed during the lipoxidase catalyzed oxidations through the reaction of free radicals. Since such polymers could possess some diene conjugation without containing hydroperoxide groups, their formation would in part explain the apparent high molecular extinction coefficient generally attributed to monomeric monohydroperoxides.

From the present studies the following conclusions were drawn: 1. The kinetics of lipoxidase catalyzed oxidation of sodium linoleate conform to the characteristics of an enzyme reaction. 2. The initially produced hydroperoxides in lipoxidase catalyzed oxidations at 0° C. contain a cis,trans diene conjugated configuration of double bonds. 3. Polymers are produced in the early stages of lipoxidase catalyzed oxidations at 0° C.

Inasmuch as the detailed results of the photo-chlorophyll oxidations will be published shortly, the major findings are merely summarized here. In the kinetic studies with and without intermittent radiation, it was found that the extent of oxidation was related only to the total amount of light absorbed, and was in no way dependent upon whether the illumination was constant or intermittent. This constitutes very strong evidence that the photo-chlorophyll oxidation is not a chain reaction like the autoxidation. Instead, an activated chlorophyll molecule appears to be involved in every step where a peroxide molecule is formed. Although no chain reaction appears to be involved, a free radical mechanism is not precluded. Any free radicals that are formed however, are either rendered so short lived or are so stabilized during their existence by complex formation with chlorophyll that no reaction chains are propagated.

That a different mechanism is involved in photo-chlorophyll oxidation is also evidenced by the chemical and physical analyses of the products formed. In the case of the photo-chlorophyll oxidation, it was found that an appreciable amount of unconjugated peroxides were formed unlike the cases of autoxidation or lipoxidase catalyzed oxidation. Further, at least some of the double bonds in the unconjugated peroxides were in the trans configuration. Further, the conjugated hydroperoxides formed were entirely in a trans,trans configuration even though the oxidation had been conducted at -1 to -2° C. This finding also indicates a mechanistic difference from autoxidation, inasmuch as the conjugated peroxides formed in low temperatures in the latter case are predominantly in a cis,trans configuration. However, it has previously been indicated that in autoxidation at higher temperatures, trans,trans conjugated peroxides are formed predominantly, and it appears that light may have the same effect as higher temperatures in producing an inversion of the cis double bonds in these conjugated systems. A similar effect has been observed in the photo-oxidation of methyl linoleate in the absence of chlorophyll, either with visible light or ultraviolet light.

The findings in the case of the photo-chlorophyll oxidation are not sufficiently complete to permit the formulation of a reaction mechanism in detail, but nevertheless permit the conclusion that a light activated chlorophyll molecule is required in the formation of each peroxide molecule.

Kinetics of the Decomposition of the Peroxides of Autoxidized Linoleate and Oleate

The effect of temperature, peroxide concentration, and level of autoxidation was determined on the rate of decomposition of the peroxide fraction of autoxidized methyl linoleate. Ultraviolet spectral and chemical changes (Table 6) also were observed at various degrees of decomposition. The results of these studies are shown in Figures 10, 11, and 12. Studies on the effect of peroxide concentration and level of autoxidation on the rate of oleate peroxide decomposition also were made (Figure 13 and 14). Also, chemical and ultraviolet changes were followed on the decomposition of oleate peroxides (Figure 15, Table 7).

In addition to the foregoing studies, such factors as the effect of added decomposition products on the rate of linoleate peroxide decomposition (Table 9), and the effect of unoxidized linoleate and linoleate peroxides on the decomposition of oleate peroxides (Table 10) were observed.

The results of this study are not in accord with the general assumption that the decomposition of fatty peroxides is a second order reaction. Even though the initial rate of decomposition is not directly proportional to the initial concentration as required for a first order reaction, the logarithm of the peroxide concentration for any given initial concentration assumes a linear relation with the time of the decomposition reaction (Figure 16). The decomposition reactions are extremely complex, due to the incidence of a number of secondary reactions. Nevertheless, it has been established that some decomposition product (s) catalyzes the decomposition, and further, that polymeric products are formed. These and other kinetic and chemical findings indicate that the decomposition involves free radical chain mechanisms, in accord with similar conclusions reached by Bateman and coworkers (1, 2).

A New Colorimetric Method for the
Estimation of Fatty Peroxides

There are a large number of methods available for the estimation of organic peroxides and no attempt will be made here to discuss their advantages and disadvantages. It has been our experience that the iodometric method as proposed by Wheeler et al. (27), and modified by Lundberg et al. (18) is the best for general use.

The method under consideration here resulted from the observation that stannous chloride appears to quantitatively reduce peroxides with a minimum of side or complicating reactions. Hydroperoxides, for example, are reduced quantitatively to hydroxyl groups under very mild conditions.

A new volumetric method involving the use of stannous chloride is finding wide acceptance in Europe. This method, while precise and having the advantage of measuring all types of peroxides, is highly complicated and time consuming.

The method proposed here consists of reacting the peroxides homogeneously in an organic solvent (ethyl ether, alcohol, acetone, or ethylene dichloride) with a measured excess of stannous chloride. The amount of unreacted stannous chloride is determined by colorimetry.

Estimation of the unreacted stannous chloride is accomplished by reduction of the dye 2,6 dichlorophenolindophenol. However, it was found that an additional reagent was needed for quantitative reaction, and oxalic acid was found to be a suitable reagent. Further, it was found that the dye inhibits action of atmospheric oxygen on the stannous chloride in the presence of peroxides. Thus, in actual practice, the reduction of the peroxides by the stannous chloride reagent is carried out in the presence of the dye. Then, after the peroxides are reduced, which requires about 15 minutes, oxalic acid is added, and the unreacted stannous chloride estimated by the decrease in the intensity of the color of the dye in a photoelectric colorimeter.

The rate of reduction of the dye by stannous chloride is shown in Figure 17, and conformance of the reaction to Beer's Law is shown in Figure 18. However, certain details of the procedure have yet to be worked out before the method can be regarded as completely satisfactory.

The chief advantages of the method are believed to be in its sensitivity and the fact that equipment for providing inert atmosphere and a constant temperature water bath is not required.

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Table 1. Analyses of Oleate Peroxide Concentrates

Analyses	Concentrate I (Original PV 5360) Reduced	Concentrate II (Original PV 5300) Reduced	Dist. Monomer
Peroxide value (m.e./kg.)	50	38	0
Iodine number (Woburn)	82.6	80.5	
Hydroxyl (moles/mole of ester)	1.05	0.96	0.99
Acid number (mg. KOH/g.)	1.5		0
Epoxy oxygen (moles/mole ester)	0.05		
Molecular weight		314	
Hydrogenation (moles/mole ester)		.65	0.63
Carbonyl oxygen (moles/mole ester)	0.12		
% Monomer		97.9	
% Dimer		2.1	
% High polymer		0	

Table 2. Analyses of Methyl Linoleate Peroxide Concentrate

Analyses	Reduced concentrate	Distilled monomer
Peroxide value (m.e./kg.)	118	0
Molecular weight	302	
Hydroxyl (moles/mole ester)	0.95	1.00
k at 234 mu	79.1	87.4
Hydrogen absorption (moles/mole ester)	1.55	1.83
Iodine value (Woburn)	170.3	
% Monomer	92.6	
% Dimer	5.6	
% Higher polymer	1.8	

Table 3. Analyses of Methyl Linolenate Peroxide Concentrate I.

Analyses	Reduced	Unreduced
Peroxide value (m.e./kg.)	175	6000
k at 236 m mu	54.8	50.8
270	6.8	6.93
280	7.0	6.03
Iodine value (Woburn)	204	--
Epoxy oxygen %	0.23	1.48
Hydroxyl (moles/mole ester)	1.04	--
Carbonyl oxygen (%)	2.66	--
Acid value (mg. KOH/g.)	14.8	13.5

Table 4. Analyses of Combined Fractions or Reduced Linolenate Peroxide Concentrate II.

| | Fractions | | | |
	1-3	4-7	8-19	20-30
Peroxide value (m.e./kg.)	600	153	100	110
k at 234 m mu	14.6	46.1	78.7	50.7
Molecular weight	222	286	310	298
Iodine number (Woburn)	148	172	207	208
Hydroxyl (moles/mole ester)	1.07	0.91	1.08	0.82
% Monomer	72.2	77.6	92.8	
% Dimer	17.8	15.7	4.5	
% Higher polymer	10.0	6.7	2.7	
% of total oxidized fraction	21.7	28.1	44.0	6.2

Table 5. Analyses of Selected Fractions from Methyl Linolenate Peroxide Concentrate II and Concentrate III.

| | Concentrate II | | Concentrate III | |
Analyses	Reduced fractions 8-19	Distilled monomer Red. fr. 8-19	Reduced fractions 9-22	Unreduced fractions 9-22
Peroxide value (m.e./kg.)	100	0	159	6100
k at 234 m mu	78.7	83.0	78.1	72.3
Molecular weight	310		314	292
Hydroxyl (moles/mole ester)	1.09	1.0	0.97	
Iodine number (Woburn)	207	229		
Hydrogenation (moles/mole ester)		2.74	2.8	3.45
% of total oxidized fraction	44.0	40.5	39.7	39.7

Table 6. Chemical Analyses of Autoxidized Methyl Linoleate at Various Stages of Decomposition

Reaction time	Peroxide Value m.e./kg.	Hydroxyl %	Acid (mg./g.)	Iodine value (Woburn)
0	1931	2.38	3.28	166.0
12	1260	2.82	6.30	159.0
24	842	2.24	6.26	154.5
36	576	2.77	4.68	124.5
48	490	2.89	4.30	153.0
72	276	-	3.92	-

Table 7. Chemical Analyses of Autoxidized Methyl Oleate at Various Stages of Decomposition (Original P.V. 1045 m.e./kg.)

Peroxide value (m.e./kg.)	Hydroxyl %	Acid (mg./g.)	Iodine value (Woburn)
1045	2.03	13.7	79.0
730	1.35	10.0	78.0
506	.83	10.2	79.0
327	.62	10.4	78.0
200	.67	10.5	76.5

Table 8. Analyses of Peroxide Concentrates Obtained from Lipoxidase Catalyzed Oxidation of Corn Oil Soaps at 0° C. and Sodium Linoleate at 26° C.

Analyses	Methyl Esters from corn oil soaps		Methyl Esters from Sodium Linoleate	
	Reduced	Unreduced	Reduced	Unreduced
Peroxide value (m.e./kg.)	74	3640	117	5010
k at 234 m mu	57.6	52.6	74.1	72.0
Hydroxyl (moles/mole ester)	0.92	--	1.00	
Molecular weight	304	241	300	218
Hydrogen absorption (moles/mole ester)	1.73	2.22	1.9	2.27
% monomer	78.5			
% Dimer	16.2			
% higher polymer	4.0			

Table 9. The Effect of Decomposition Products on the Rate of Decomposition of Linoleate Peroxides

Sample	P. V.	Rate %/hr.	% decomposed peroxides	Percent increase in rate	Theoretical P. V.	Acid Value (% linoleic acid)
1	458	1.07	none	---	458	nil
2	455	1.20	2.16	12.1	585	0.55
3	450	1.26	4.0	17.7	695	0.79
4	453	1.34	5.8	25.1	826	0.90
5	450	1.47	10.5	37.4	1074	1.15

Table 10. The Effect of Oxidized and Unoxidized Methyl Linoleate on the Decomposition of Autoxidized Methyl Oleate at 80° C.

Sample		Treatment	P.V.	Rate (%/hr.)
1		None	1045	0.46
2	+	2.3% unoxidized linoleate	1000	0.44
3	+	1.05% oxidized linoleate (P.V. 1000)	1032	0.45
4	+	2.5% oxidized linoleate (P.V. 1000)	1030	0.45

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Figure 1.

Ultraviolet Spectra

Oleate Peroxide Concentrate I
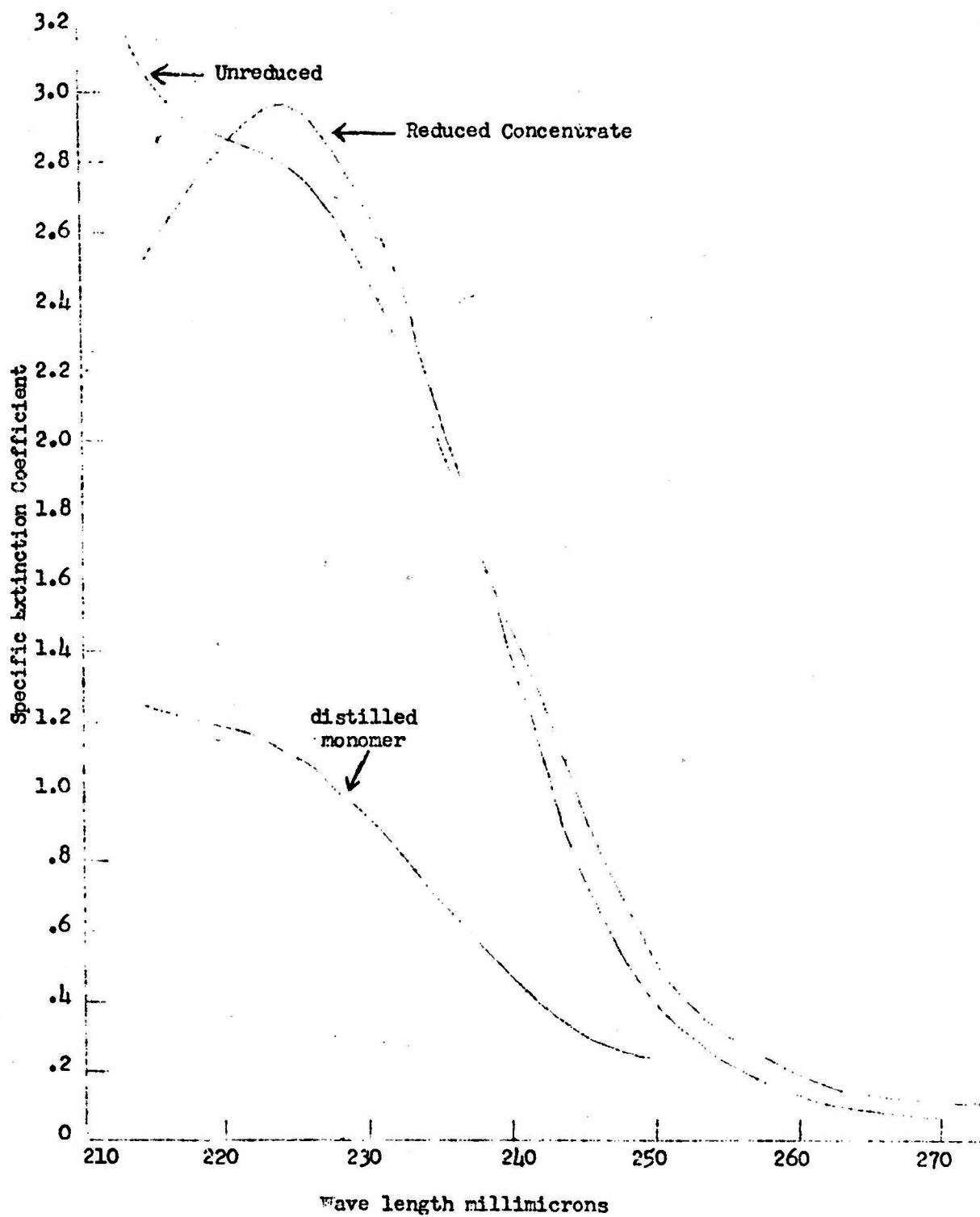
Figure 2.

Infrared Spectra

Oleate Peroxide Concentrate I
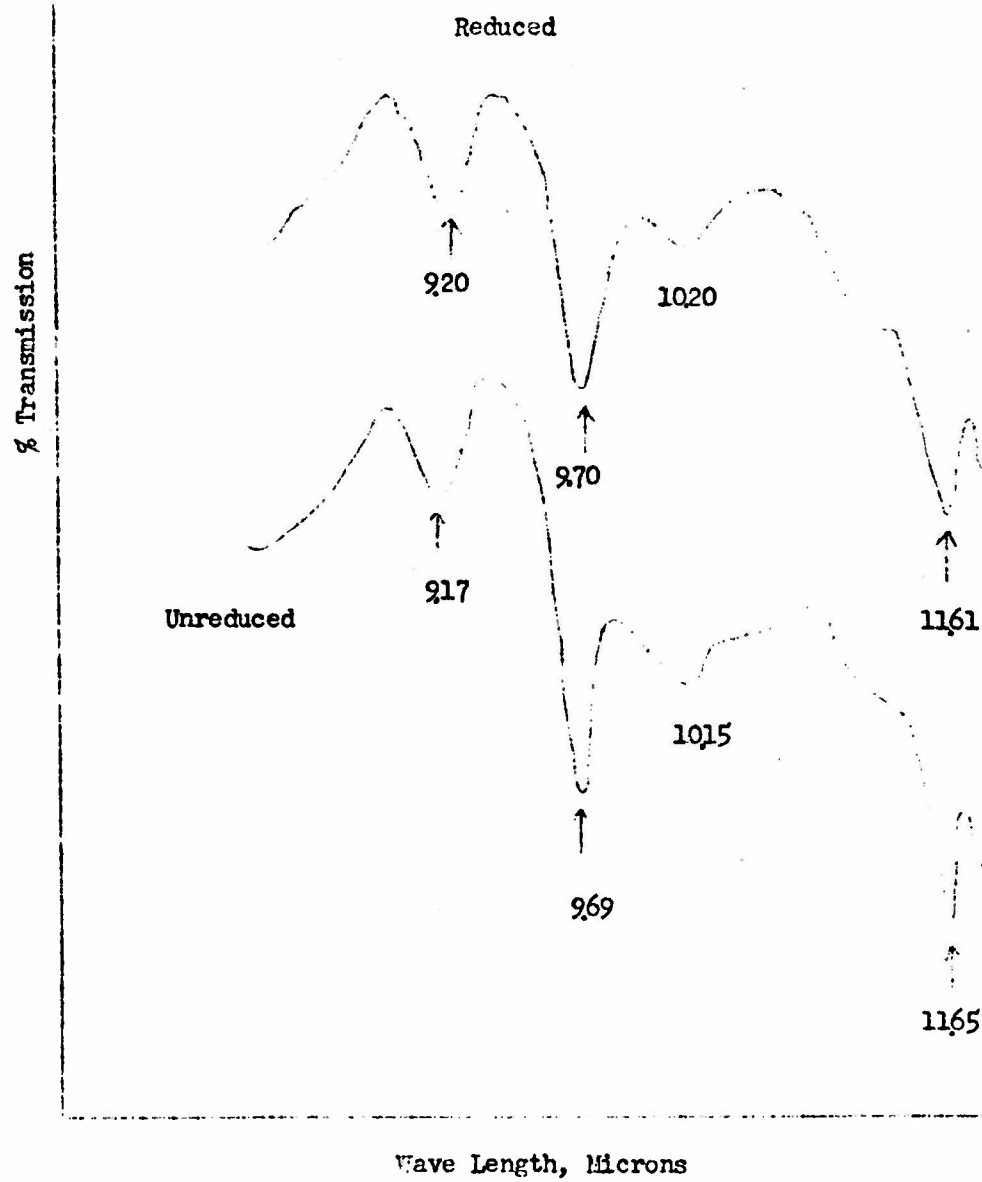
Figure 2A
Infrared Spectra
Oleate Peroxide Concentrate I
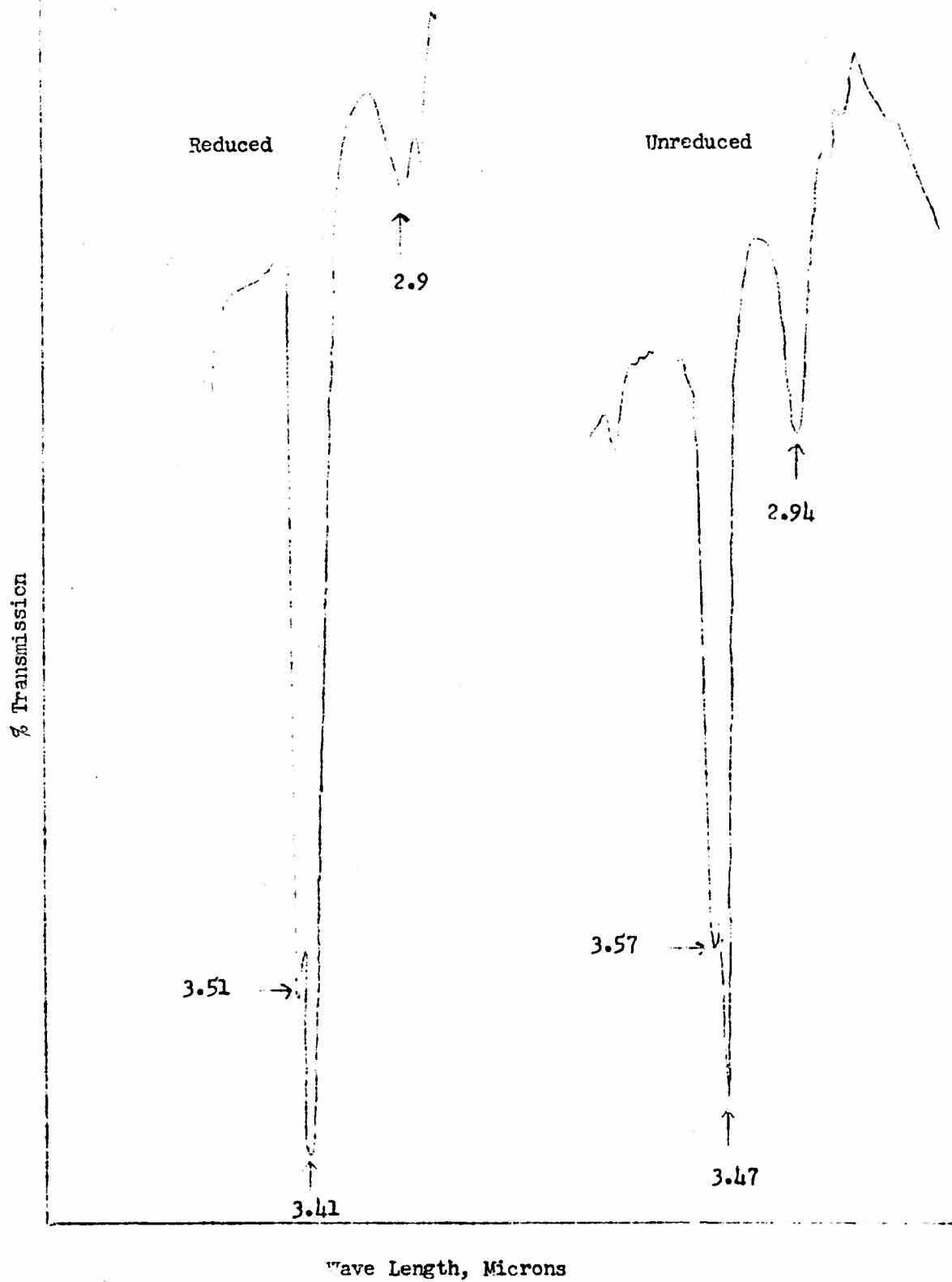
Figure 3

Infrared Spectra

Reduced Linoleate
Peroxide Concentrate
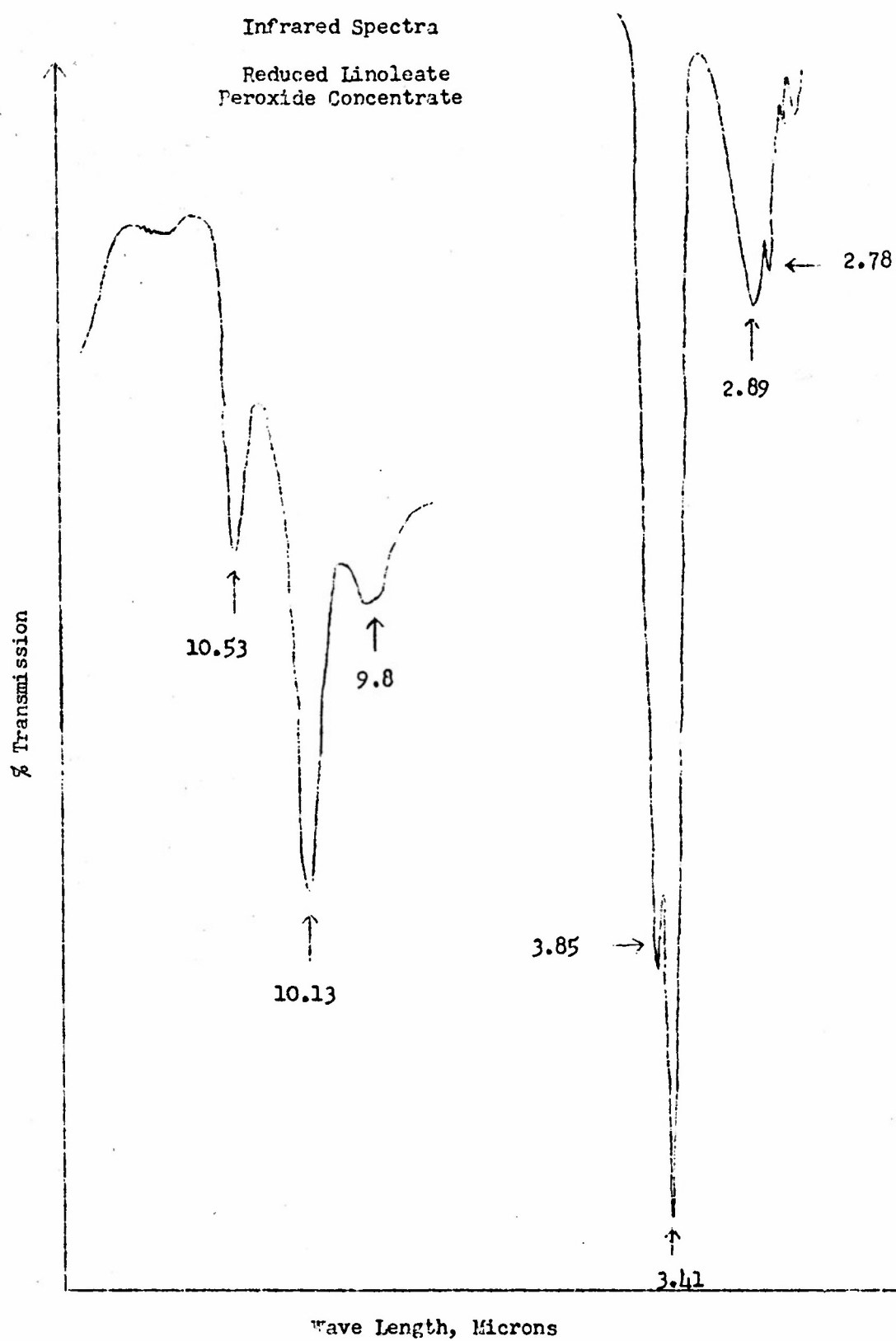
Figure 4
Linolenate Reduced Peroxide Concentrate II.

Ultraviolet spectra:
A – Combined fractions 8-19
B – Combined fractions 4-7
C – Combined fractions 1-3
D – Original autoxidized ester
E – Recovered unoxidized ester

Specific Extinction Coefficient, k

WAVELENGTH, mu

Fig. 5   PEROXIDE VALUE ME./KG.
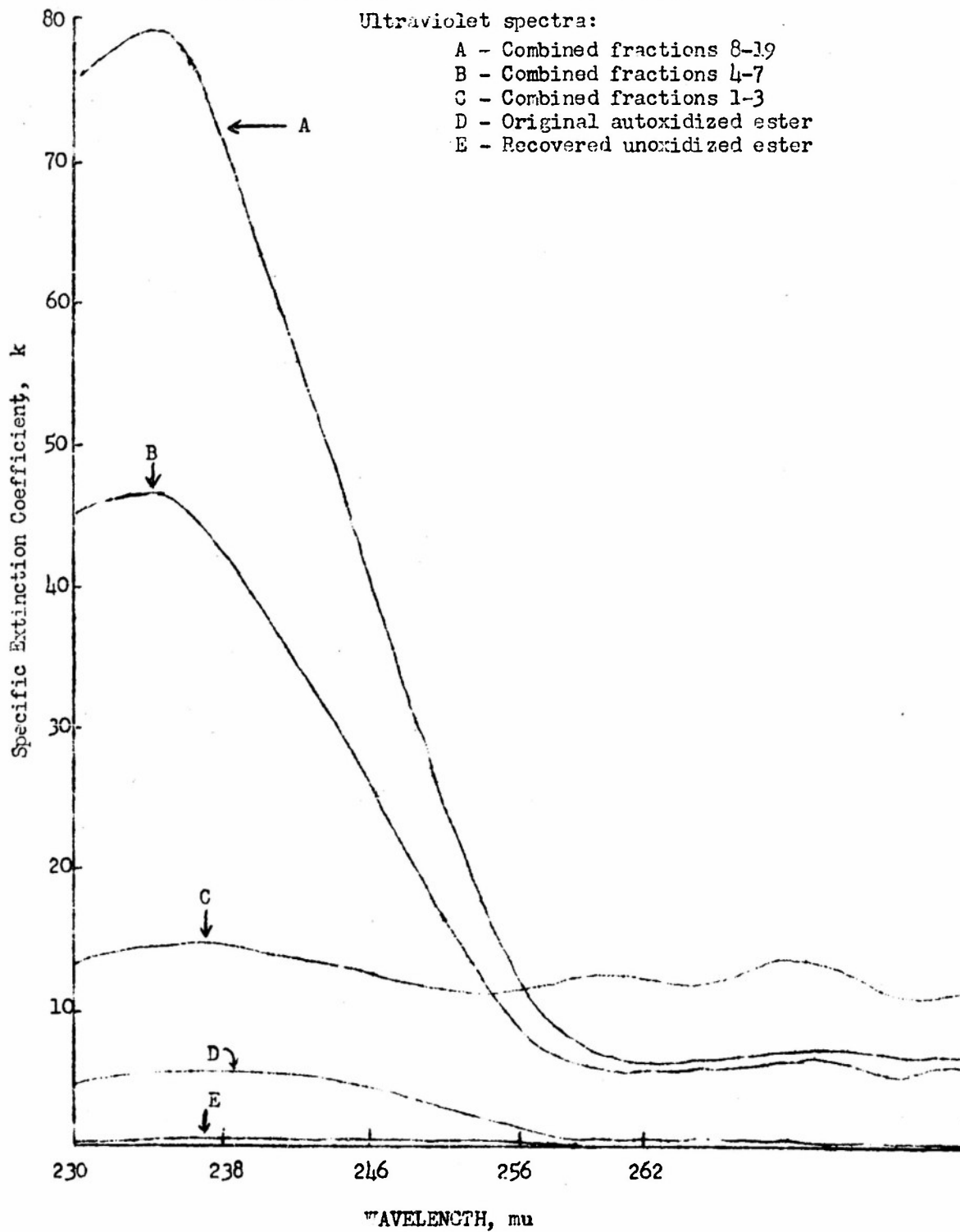
Distribution Analyses – Methyl Linolenate Concentrate II.

k, 236 mu

Peroxide value

k at 236 mu

% of total oxidized material

22%   28%   47%   6%
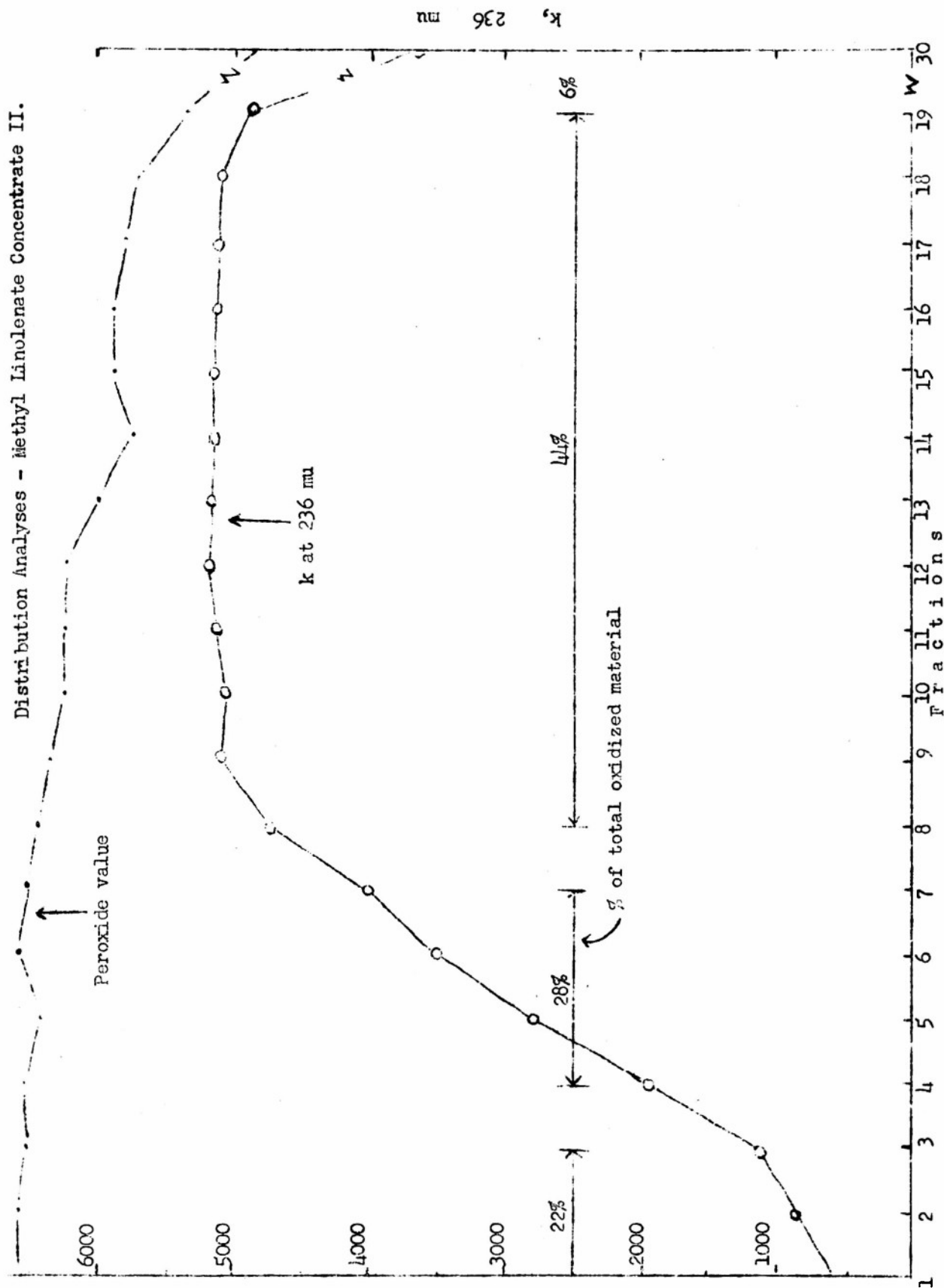
Figure 6
Methyl Linolenate Peroxide
Infrared Spectrum Concentrate I (10% solution of CS_2)

Figure 7

Infrared Spectra:

A. Unoxidized methyl linolenate
B. Recovered unoxidized methyl linolenate
C. Distilled monomer of fractions 8-19, linolenate reduced concentrate II.
D. Distilled monomer of methyl linoleate reduced concentrate.
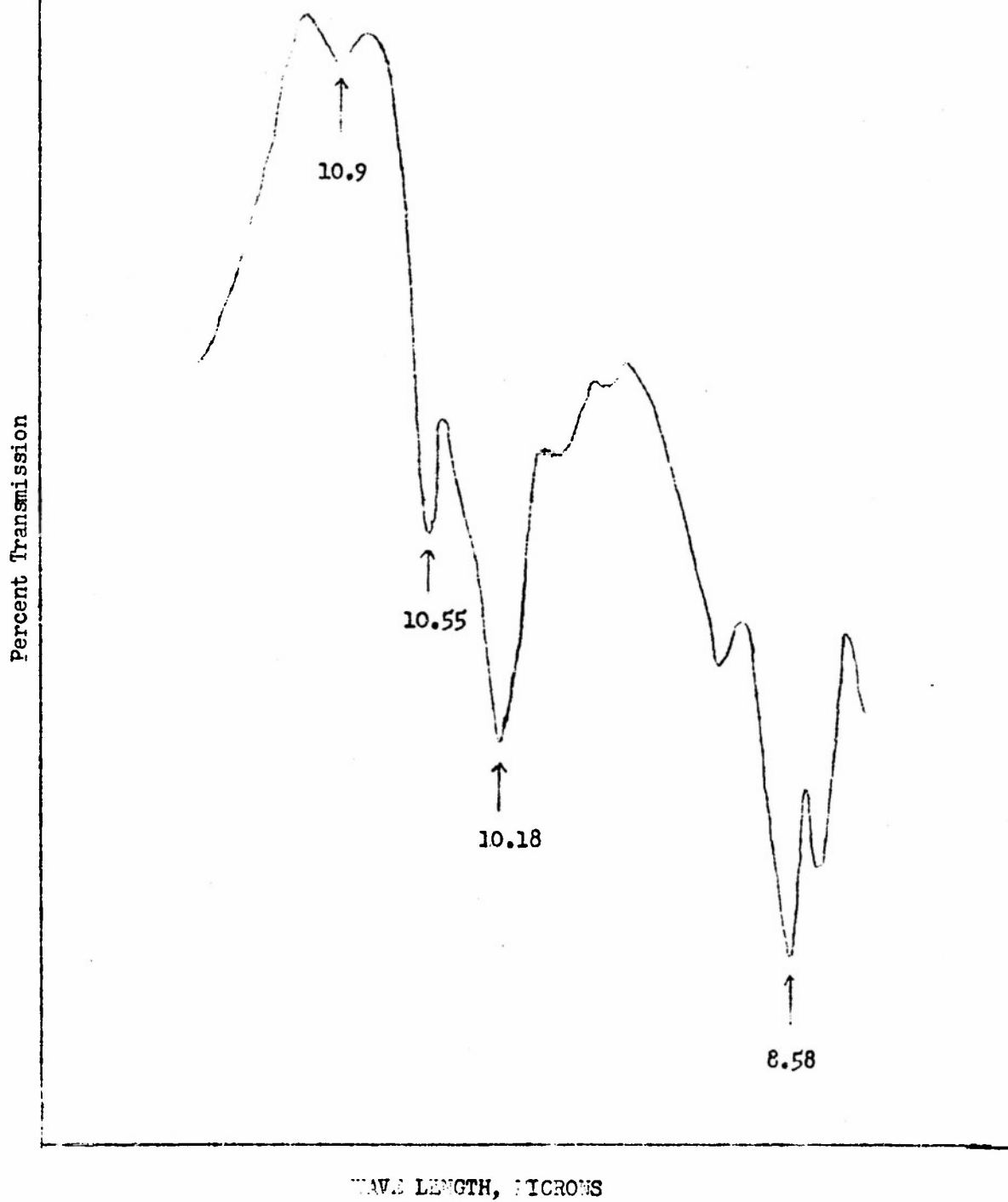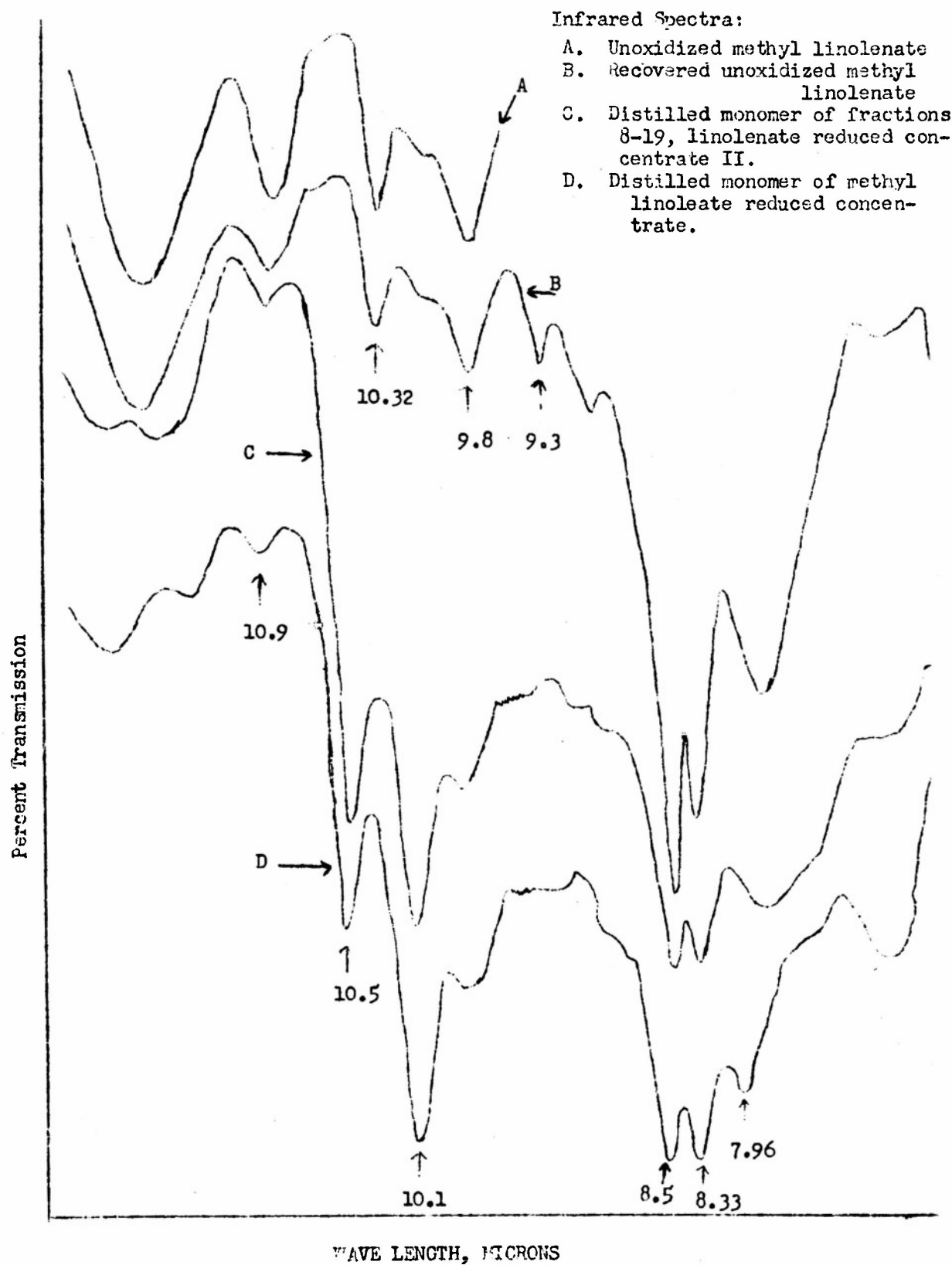
Figure 8
Infrared Spectra. Selected combined fractions of reduced linolenate concentrate II
(10% solution in CS_2)
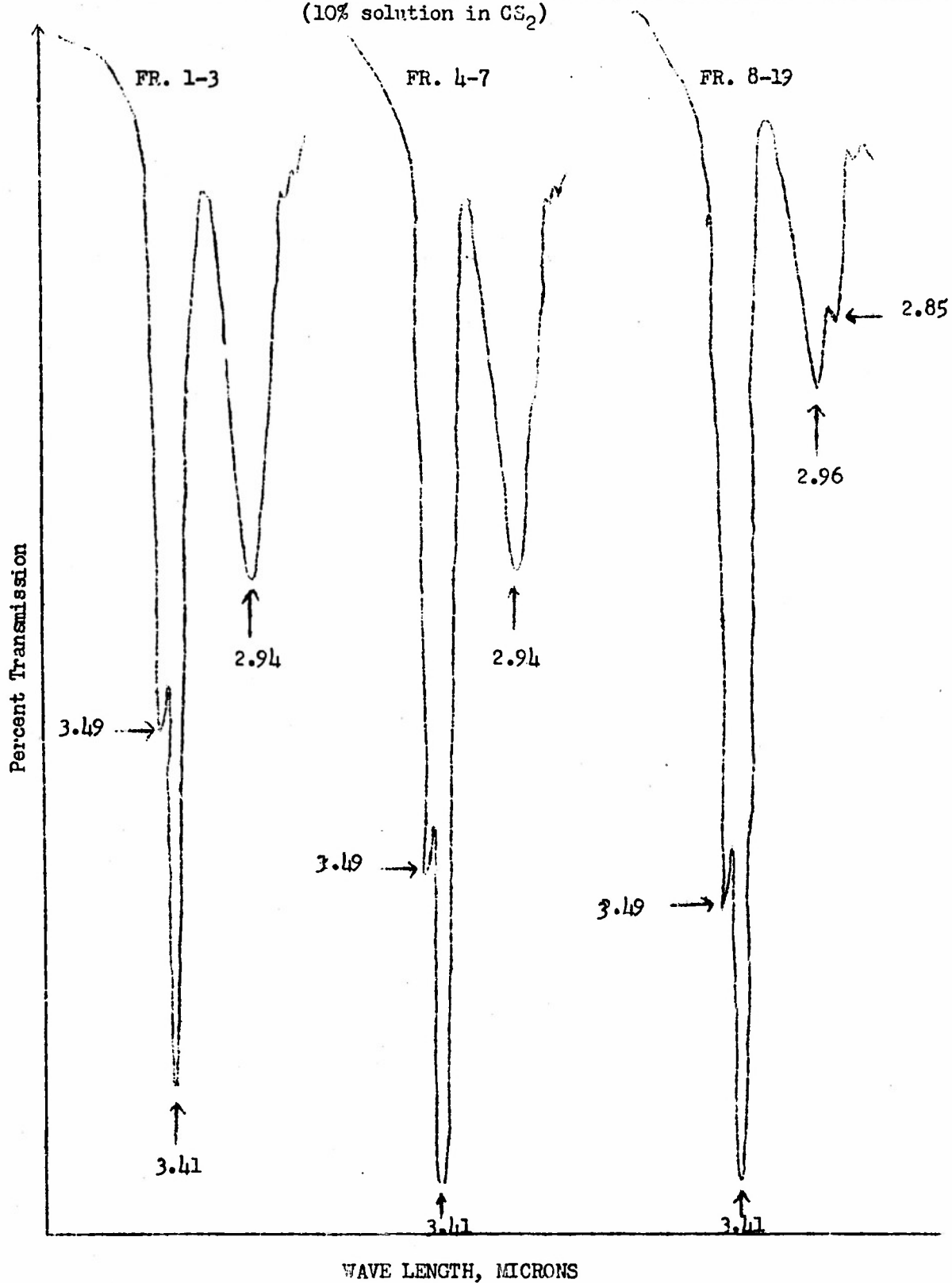
Figure 9
Infrared Spectra. Selected combinations of fractions of reduced linolenate peroxide concentrate II (10% solution in CS_2).

PERCENT TRANSMISSION

FR. 1-3

FR. 4-7

10.92

FR. 8-19

10.52

9.85

9.0

7.64

8.04

10.17

8.33

8.55

WAVE LENGTH, MICRONS
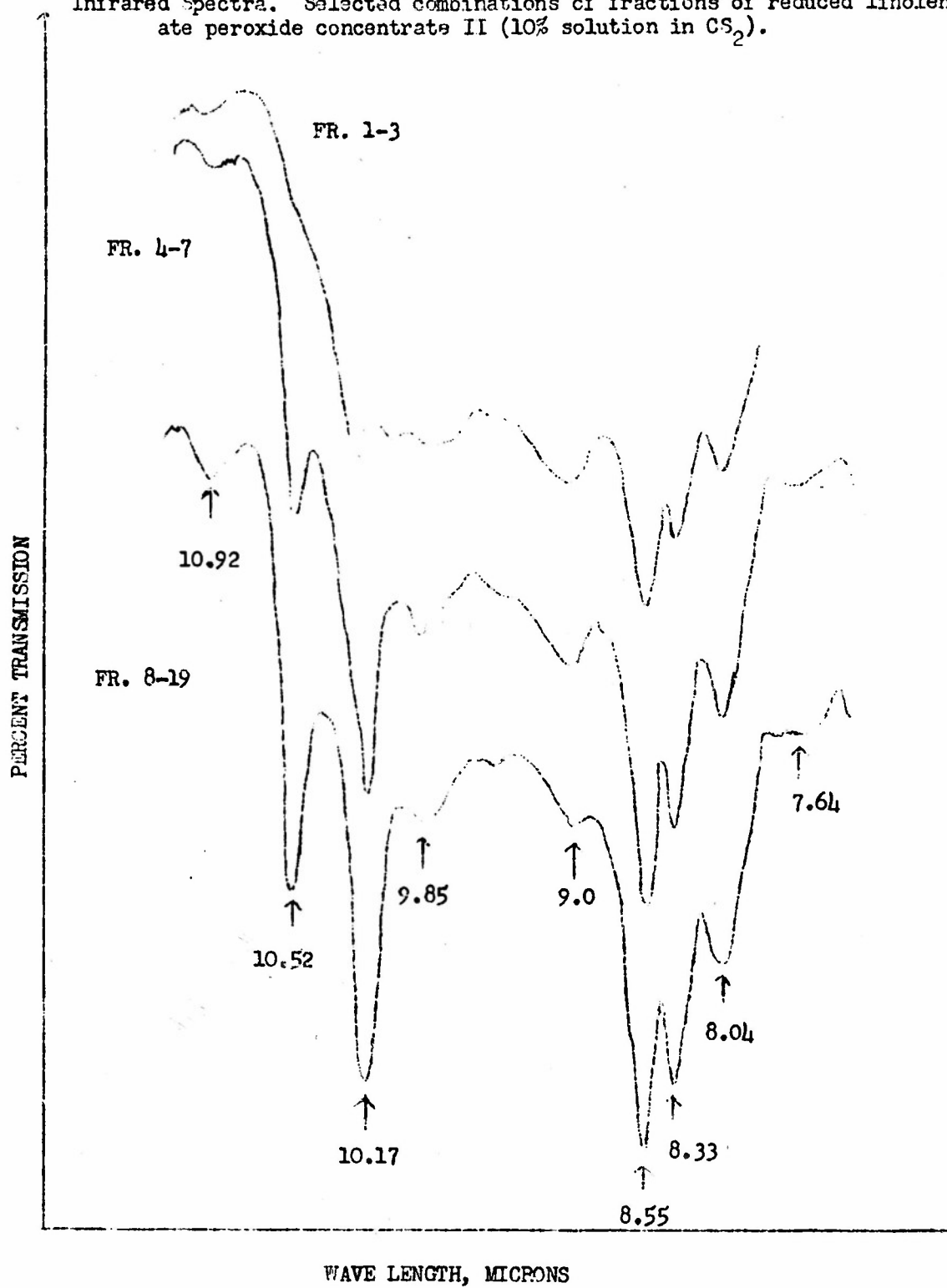
Figure 10

Ultraviolet Absorption Spectra of Autoxidized
Methyl Linoleate (PV 951) at Various
Stages of Decomposition at 80° C.

A – original
B – after 23 hours
C – after 47 hours
D – after 68.7 hours
E – after 92.5 hours
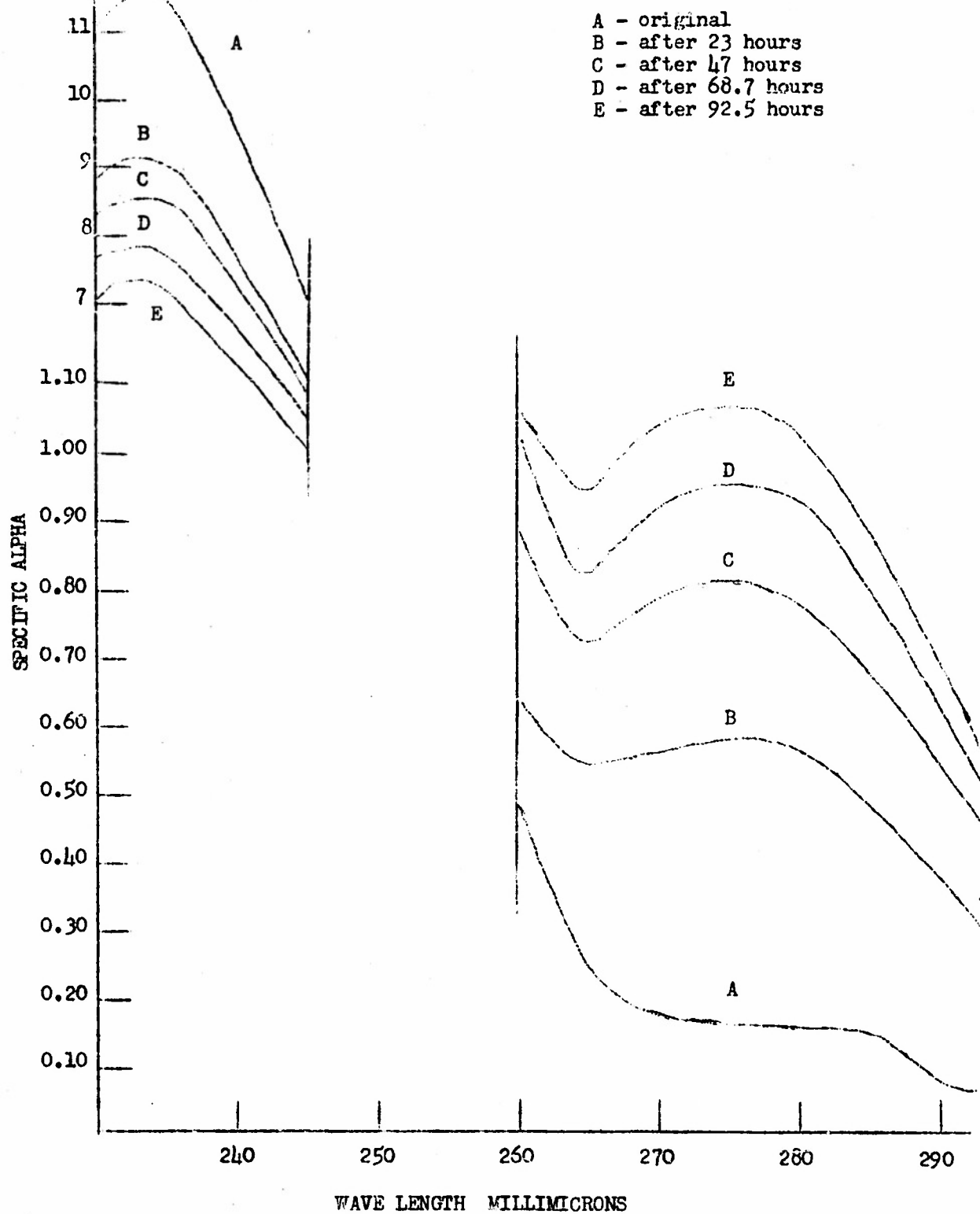
Figure 11

Effect of Temperature on the Decomposition
of
Autoxidized Methyl Linoleate
PV 1081
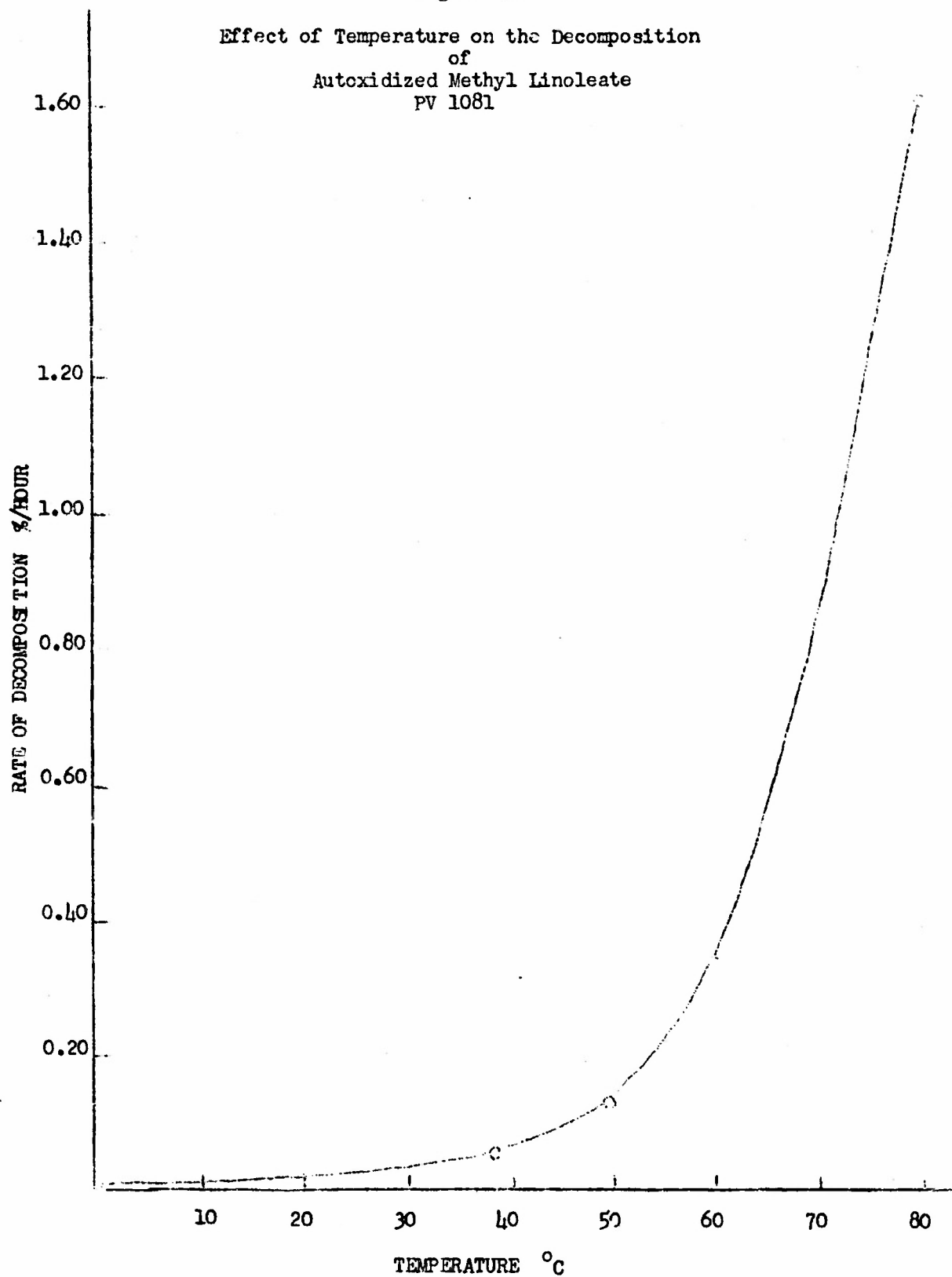
Figure 12

Effect of Level of Autoxidation on the Rate

of Decomposition of Linoleate Peroxides
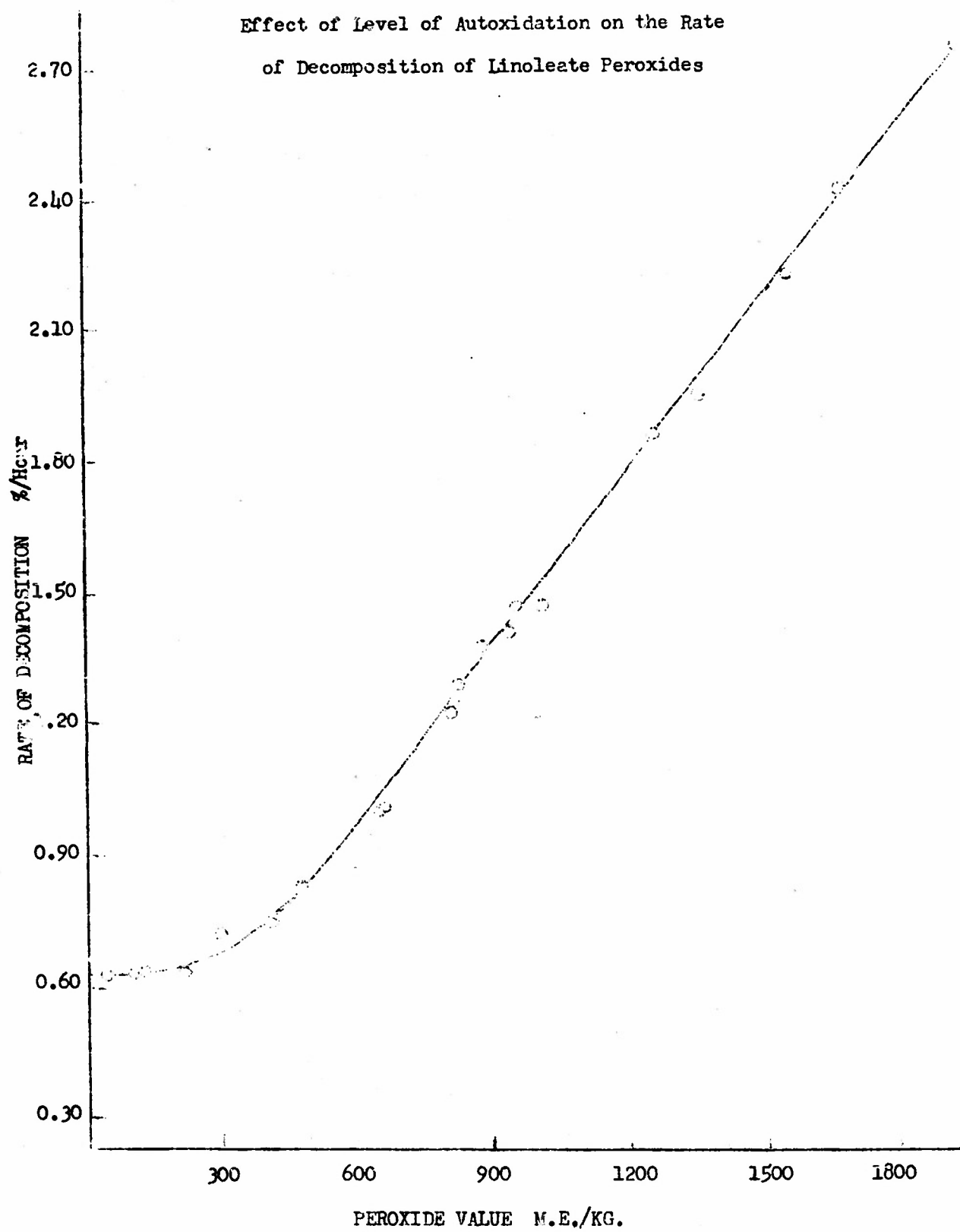
Figure 13

Effect of Level of Autoxidation on the Rate
of Decomposition of Oleate Peroxides
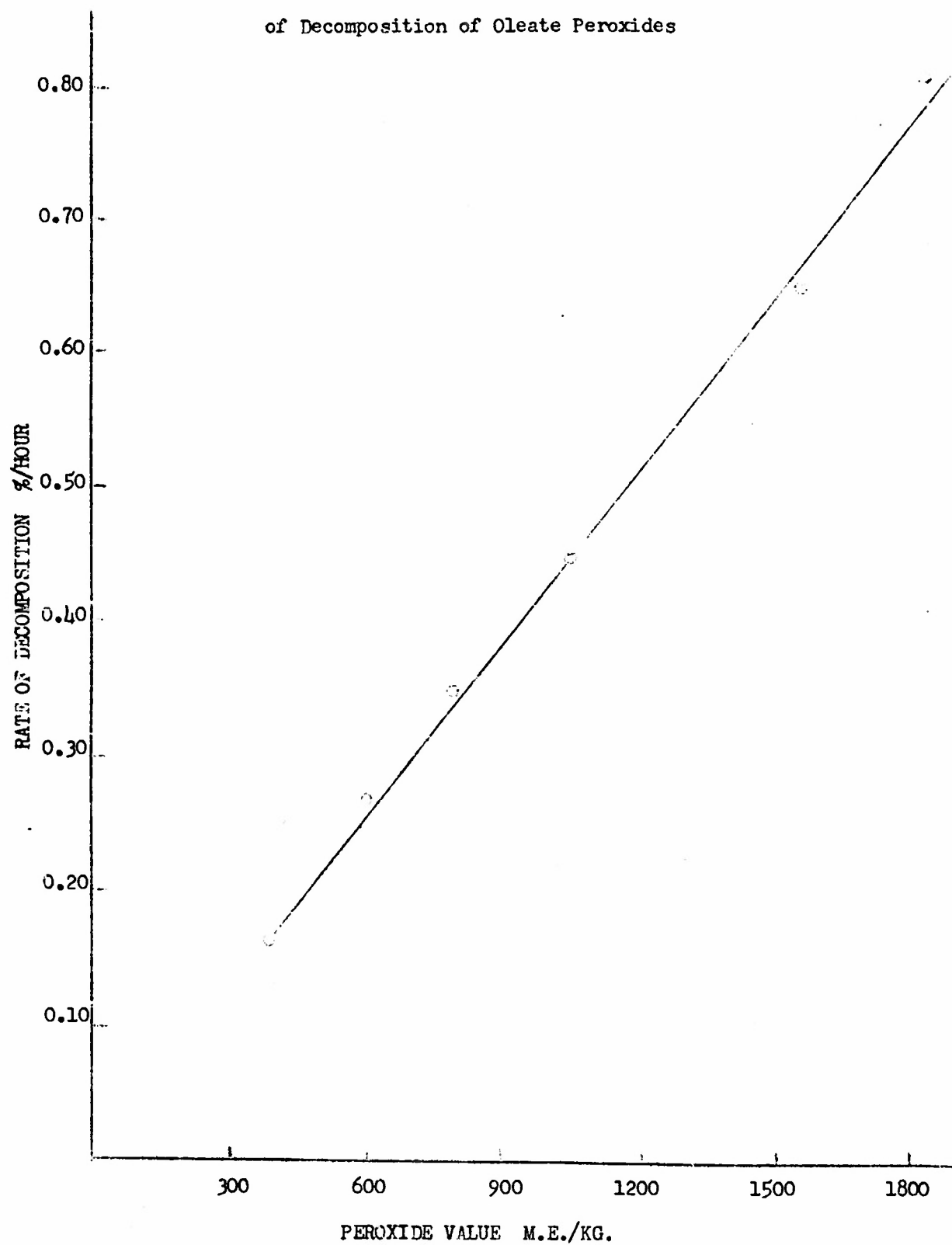
Table 14

Effect of Concentration on the Rate
of Decomposition of Oleate Peroxides
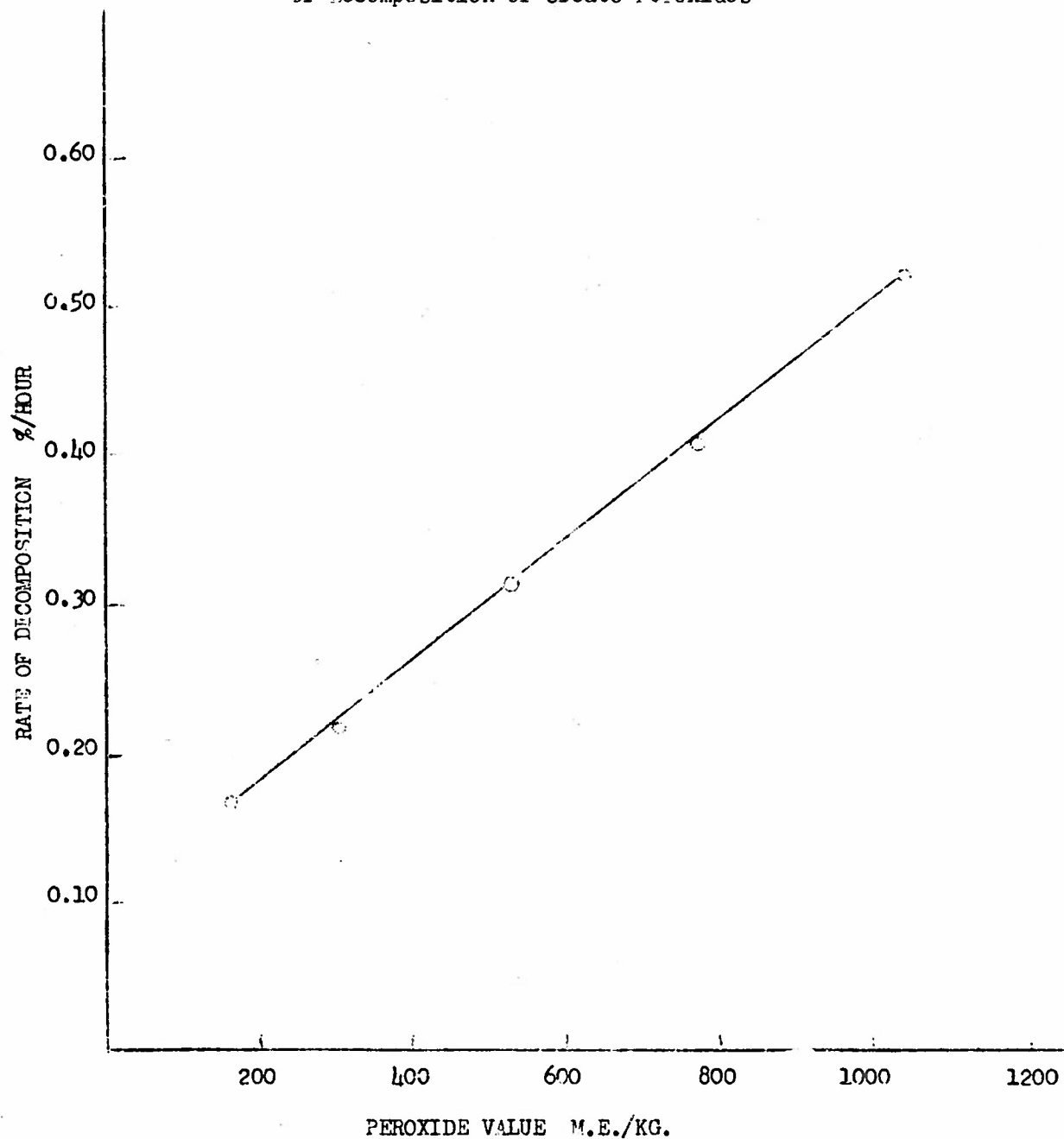
Figure 15

Ultraviolet Absorption Spectra of Autoxidized
Methyl Oleate at Various Stages
of Decomposition at 80° C

A – original
B – after 24 hours
C – after 51.4 hours
D – after 119.2 hours
E – after 198 hours
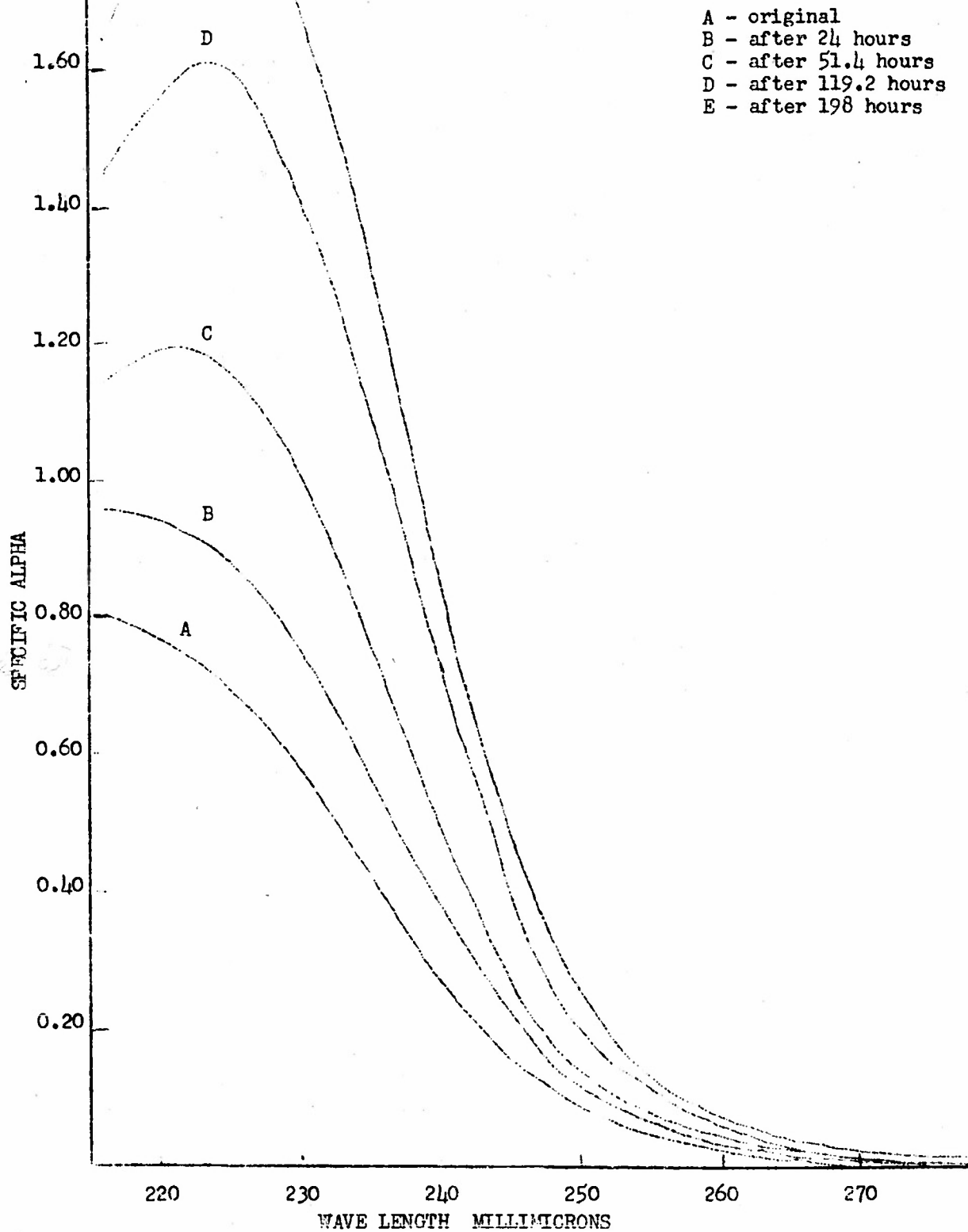
Figure 16

The Rate of Decomposition of Autoxidized
Methyl Linoleate   PV 951 at 80° C
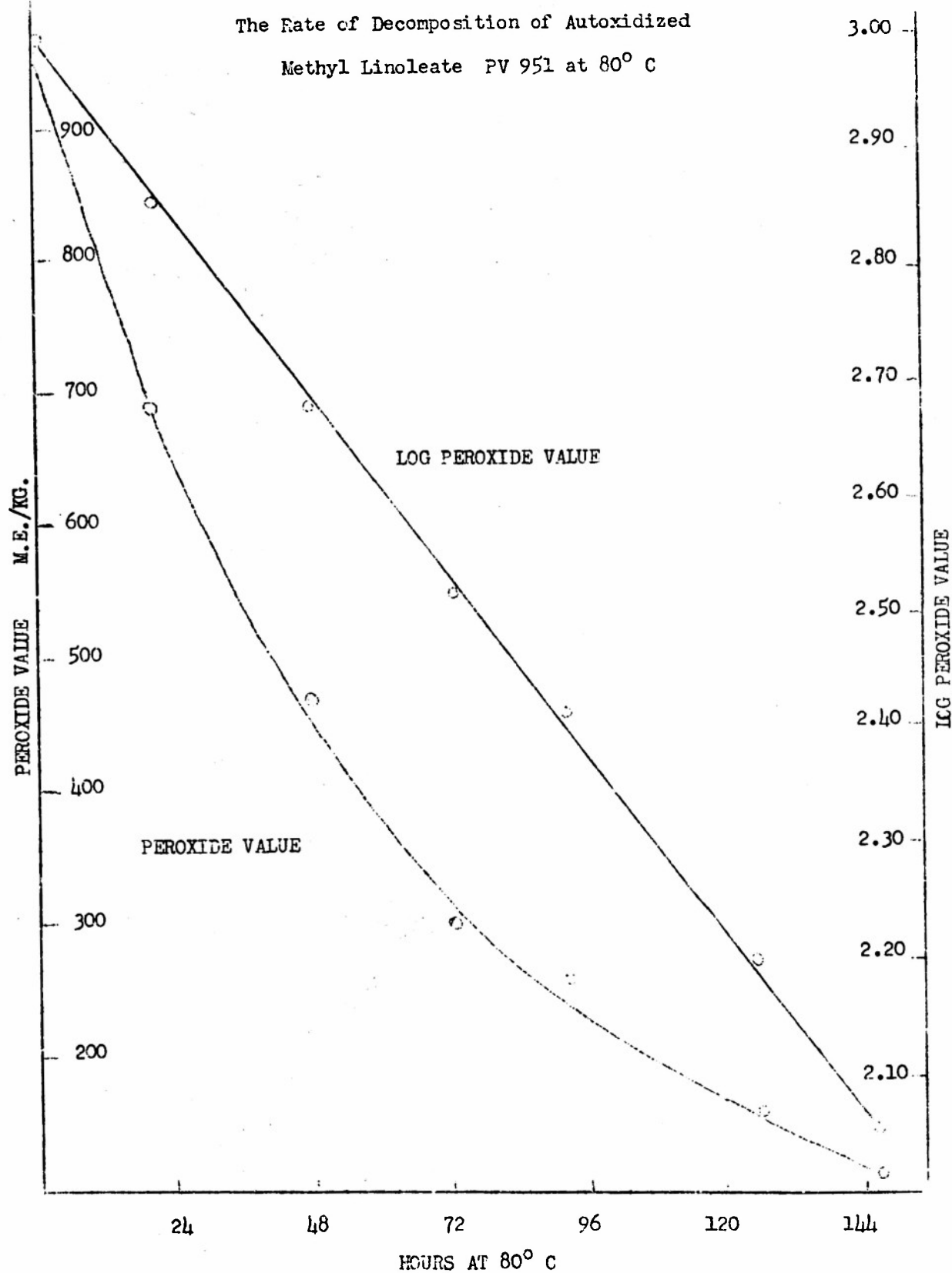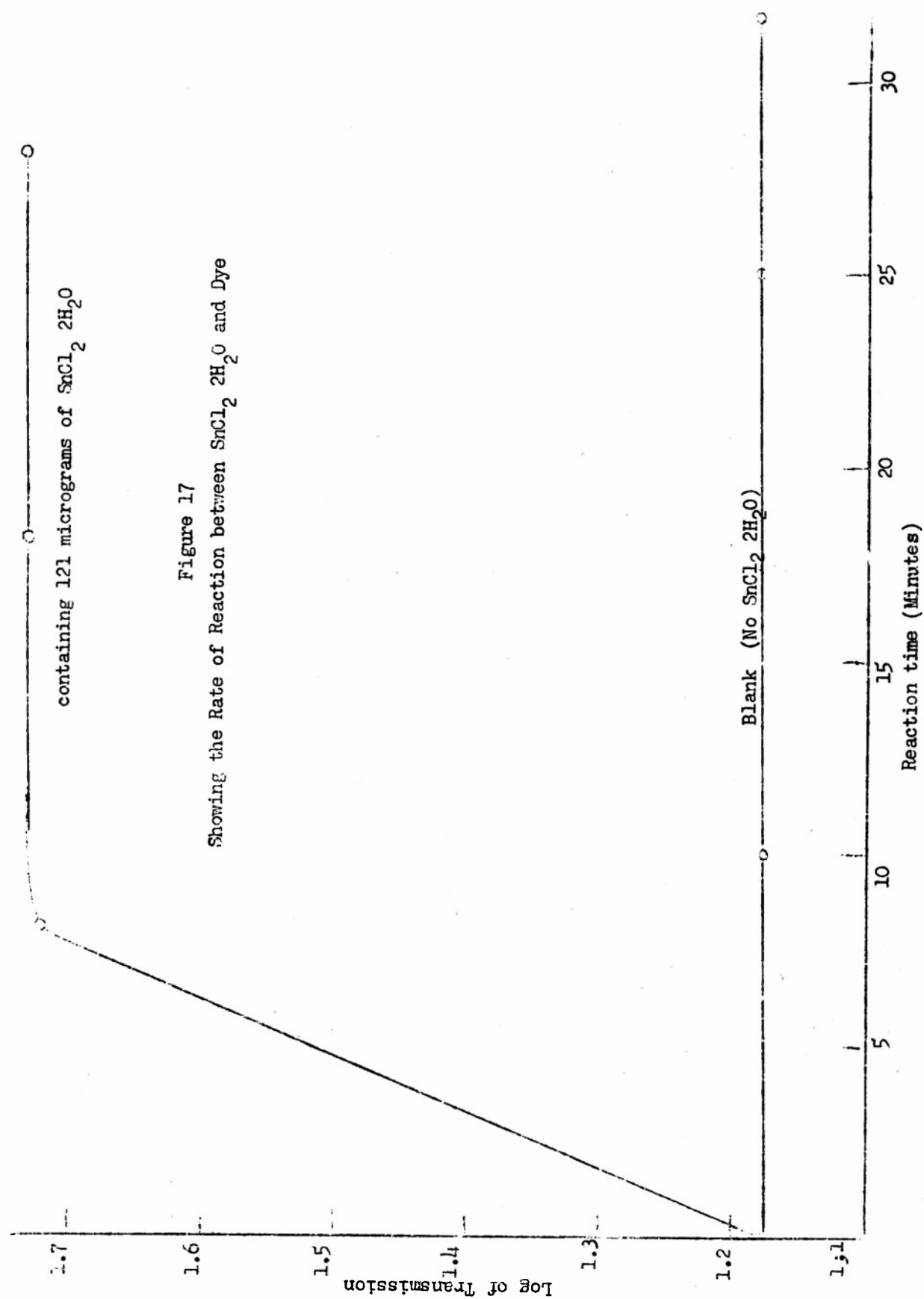
Figure 17

Showing the Rate of Reaction between $SnCl_2 \cdot 2H_2O$ and Dye

Figure 18. Standard Curve for Stannous Chloride
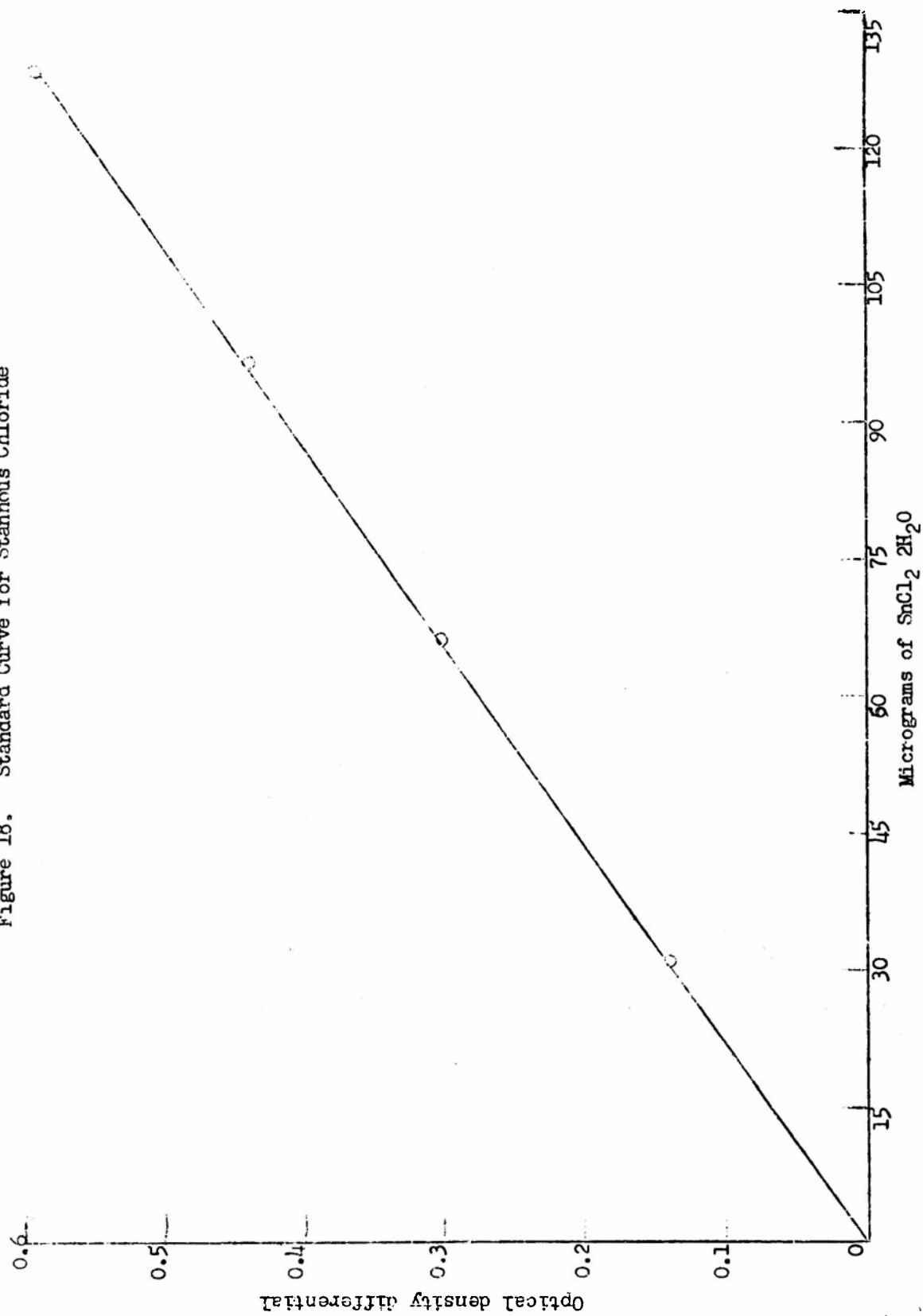
Figure 19

Infrared Spectra

Wave Length, Microns
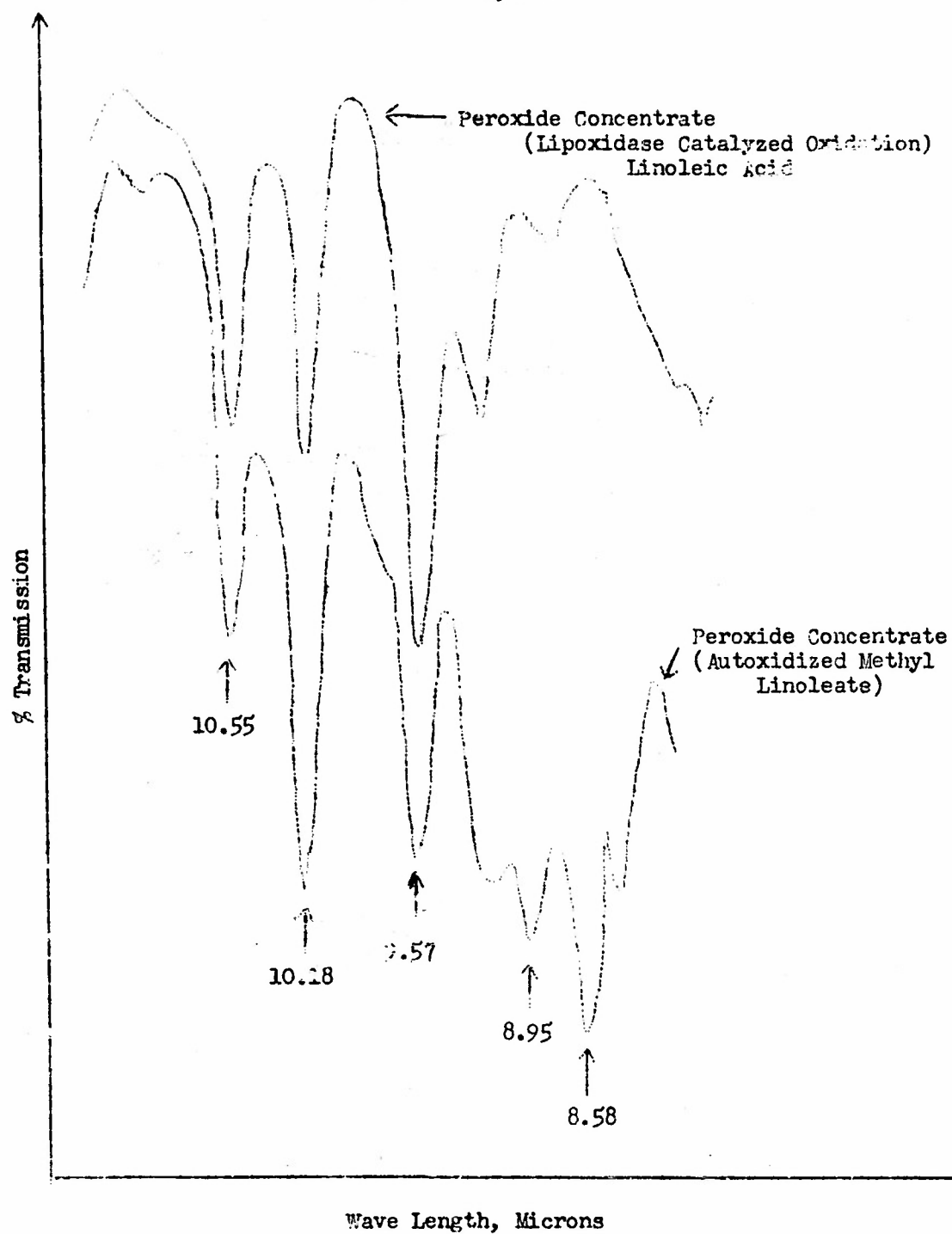
Figure 20

Infrared Spectra

Peroxide Concentrate Fractions from Corn Oil

Acids Oxidized in Presence of Lipoxidase
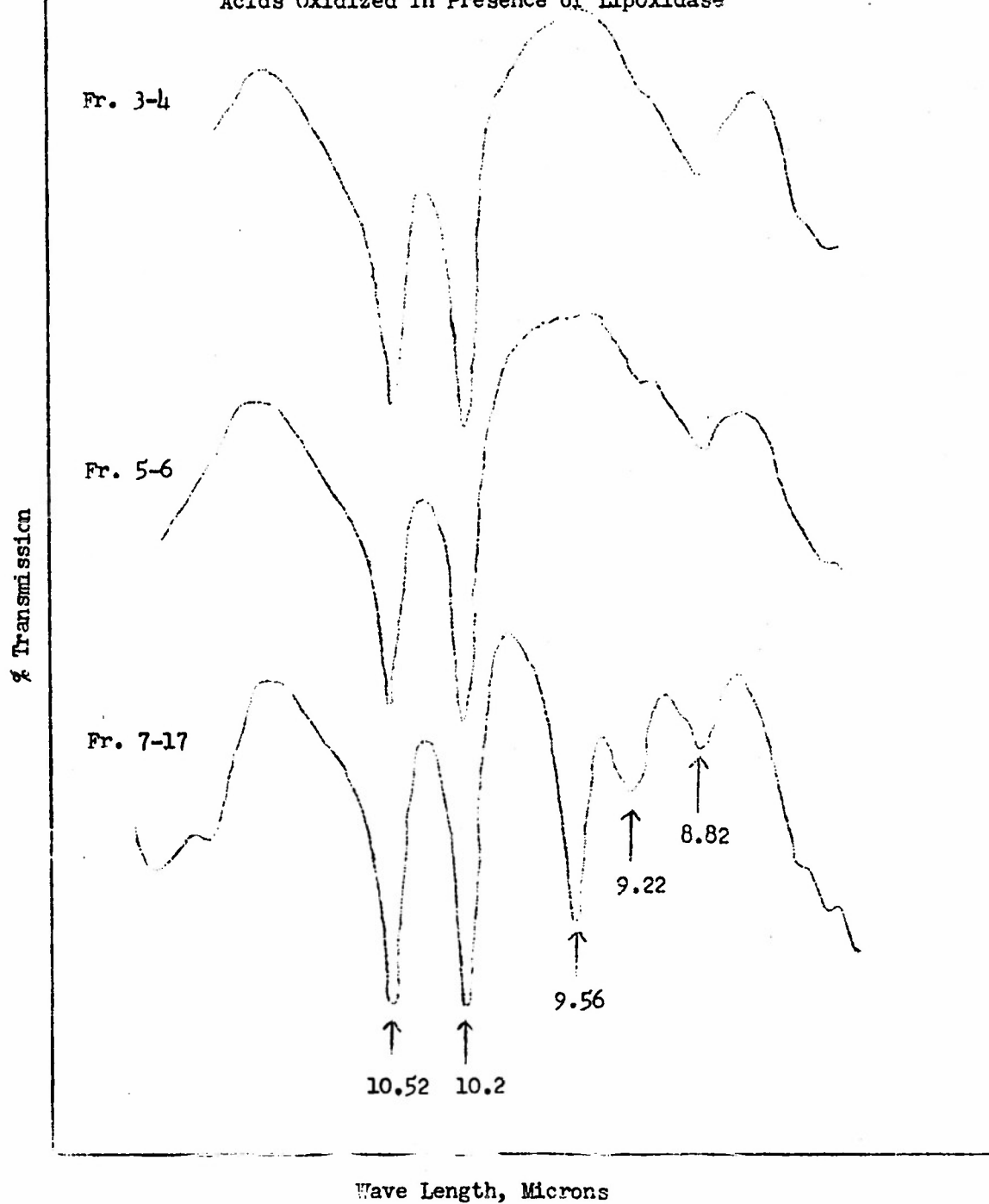
Figure 21

Ultraviolet Spectra

A - Peroxide Concentrate (PV 3640)
    obtained from lipoxidase catalyzed
    oxidation of corn oil soaps.

B - Original oxidized corn oil soaps
    PV 635 (lipoxidase catalysis)

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